University of Alberta
Printing Department

THE UNIVERSITY OF ALBERTA

THE FINE STRUCTURE OF THE ENDOCARDIUM IN ADULT RATS

BY

HAUKUR MELAX

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ANATOMY

EDMONTON, ALBERTA

MAY, 1966

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled .....

THE FINE STRUCTURE OF THE ENDOCARDIUM IN ADULT RATS

submitted by HAUKUR MELAX .....

in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Electron microscopic studies were carried out to obtain information on the ultrastructure of endocardium in normal adult rats. The specimens were chosen from the various locations of the heart chambers. Hundreds of sections from these different specimens were examined, under the electron microscope, to gain a wide range of information regarding the endocardium.

ACKNOWLEDGEMENTS

I express my thanks to Dr. T. S. Leeson for introducing me to this field of research, his constant guidance and valuable suggestions throughout the entire study. To Professor W. R. Salt a special thanks for reading through the manuscript. To Dr. D. A. Ollerich for his valuable suggestions. Technical help and other assistance by Mr. R. Adair, Miss C. Boyd and Miss M. Fogarty is appreciated. Graduate Research Assistantship from the University of Alberta is gratefully acknowledged.

TABLE OF CONTENTS

INTRODUCTION

We have no description of endocardium from Assyrians, Babylonians or other ancient peoples, but it is known that these peoples developed among themselves a considerable degree of knowledge in anatomy.

It is believed that endocardium was described by Vesalius or his followers during the Renaissance but there is no definite knowledge of their understanding of this structure.

Jean - Baptiste Bouillaud (1796 - 1881) was an outstanding clinician. He is believed to be among the first to give a satisfactory description of the endocardium. From his paper, "Traite clinique des maladies du coeur," the following excerpt has been translated (Jarko 1958). "Although it has been somewhat neglected by anatomists, the study of this membrane it is of great importance to the physician. I name it the endocardium to contrast it with the term pericardium, which refers to the external envelope of the heart." He also stated; "Neither vascular twigs nor nerve filaments are seen in the fine web of the endocardium in normal conditions."

It is possible that Bouillaud's ideas about the endocardium were influenced by those of his predecessor Marie - Francois - Xavier Bichat (1771 - 1802) whose "Traite des membranes" is one of the foundation stones of modern anatomy. However, Bichat's ideas about endocardium were quite vague. In his summary he stated; (Jarko 1959) "What is the nature of it? We have no datum respecting it; less extensible than any one of the membranes already described, it breaks by the

least effort, as we see in aneurym and in ligatures on the arteries, strongly tied. Its mode of sensibility is hitherto little known."

With the development of the light microscope and the introduction of chemicals for staining of tissues the histologists' knowledge and understanding of tissue structure were broadened enormously. In the earlier years of this exploratory age very little attention was paid to the structure of normal endocardium. However, as a result of an interest in the innervation of the heart walls, some attention was attracted to the adjacent endocardium.

Smirnow (1895) describes nerve endings in close association with the endocardium in mammals.

Woollard (1926) suggested strongly the possibility of nerve endings in the endocardium.

Holmes (1957) demonstrated nerve endings in the atrial endocardium. He states; "The endocardium of the dog atrial wall after methylene blue staining shows thick myelinated fibers which run to circumscribed end formations. These are located particularly about the point of entry of the veins, and are thought to be receptor in function. A second nervous structure consists of a more widely distributed fine fibered net and include cells, the "terminal nervous network"."

These studies obviously indicate that there are nerve endings in the subendothelial layer of the atria. However, these authors did not give any satisfactory description of the structure of the endocardium itself, nor did they demonstrate any nerve endings in

the endocardium of the ventricles.

During the last three decades, the invention and use of the electron microscope has opened up a new world of structures in cells and tissues. Considerable work has been done on the endothelium of blood vessels and the myocardium itself, but work on the fine structure of the endocardium has been largely neglected. To date only two papers describing the fine structure of normal endocardium have been published.

Candiollo (1963) was the first to describe the fine structure of the endocardial endothelium in adult albino rats. He confined his studies to the endocardium of the papillary muscle only. His electron microscopy investigations revealed that the contiguous surfaces of the endothelial cells are overlapping and are separated from one another by a space of about 100 A^{o}, which he believes is filled with amorphous basement membrane material. He found that the subendothelial layer contains collagen fibers and fibroblasts, but he did not find any nerve endings or at least they were not mentioned nor demonstrated. Candiollo states: "The ground substance is rich in clumps of an amorphous material showing similar staining behaviour and location as those of fibers which are believed to be elastic. These clumps are more numerous in the deep areas of the endocardial tunica proprix, hence close to the cardiac muscle fibres." In his summary he states: "Intensely stained are also clumps of material pinched off from the plasma membrane and released into the cavity."

Stehbens and Meyer (1965) published the second description

of fine structure of endocardium. The frog's ventricle, which consists of a spongy network of muscle trabeculae, does not have coronary circulation supplying the myocardium. The intertrabecular spaces communicate with the main central cavity. Their observations revealed overlapping of adjacent endothelial cells, but interdigitation was reported uncommon. Pinocytotic vesicles are numerous in the endothelial cells of frog's ventricle. Stehbens et al. found deficiences in the endothelial lining between cells. Most of them were less than two microns, but the largest ones measured up to eight microns in diameter. In these gaps the subendothelial layer is exposed to the circulating blood and no evidence of a diaphragm was found. In general there was no basement membrane on which the endothelial cells sit, and the subendothelial layer is often absent leaving the endothelial cells exposed to the myocardium, and at the gaps myocardium is exposed to the circulating blood. Collagen fibrils and fibroblasts are present where there is a subendothelial layer. No nerve endings were described in the subendothelial layer. The authors state, "The endothelium of the frog heart (Hyla caerulea) was examined by electron microscopy. The ventricle was avascular, blood supply being derived from blood circulating in intertrabecular spaces of the spongy myocardium. These spaces were lined by endothelium characterized by the virtual absence of basement membrane and the prevalence of gaps up to 8 microns in width."

Bennett et al. (1959) have done a great deal of work on capillaries in many organs and tissues, and their classification of

capillaries on the basis of endothelial cell types is well known. Unfortunately their work did not include a study of the endocardial endothelium.

Several electron microscopy studies have been carried out which reveal the fine structure of the aorta. The histological features of the tunica intima of the aorta in comparison with the endocardium are of interest.

Pease et al. (1960) were among the first to describe the fine structure of the thoracic aorta in normal rats. The only cell type they found in the tunica intima was endothelium. Small masses of intracellular elastin were present in the cells. The internal elastic lamina was found to be a thick sheet of elastin, with the inner surface rough. These membranes were found to be fenestrated, and in the vicinity of a fenestration a relatively large subendothelial tissue space appeared where bundles of collagen are frequently present. In their paper Pease et al. state: "Intercellular membranes are commonly elaborately interdigitated. Fairly often endothelial flaps protrude into the vascular lumen in association with these junctions."

Another report on fine structure of the normal rat aorta was given by Keech (1960). His observations on tunica intima were very much the same as those of Pease et al.

The ultrastructure of the myocardium has been revealed by several workers during the last ten years. Huxley (1961) believes that the contractile elements of the cardiac muscle are identical to those of skeletal muscle. However, a few points at which differences

occur are obvious. The most noticeable one is the large number of mitochondria in cardiac muscle. Secondly the heart muscle fibers are small in diameter in comparison with those of skeletal muscle. A more thorough description of the ultrastructure of mammalian cardiac muscle was given by Stenger et al. (1961). Sjostrand et al. (1960) revealed the fine structure of the intercalated discs, and Battig et al. (1961) studied the connective tissue space of human cardiac muscle. Unfortunately these authors did not extend their studies into the endocardium.

Techniques in the fields of electron microscopy are being continuously improved and more and more details of the structure of the cells and tissues are being revealed. To date many areas have been thoroughly investigated, while others have been neglected. Endocardium has been one of the neglected areas of study. Information on the fine structure of the endocardium in adult rats is incomplete. The fine structure of the endocardial endothelium, its cell junctions, its micropinocytotic activity, its basement membrane remain to be examined. The subendothelial layer is almost unknown. How thick is it? Are there any nerve endings in the subendothelial layers of the ventricles? Was Candiollo right when he stated that elastic clumps are present in the subendothelial layer of the ventricles or did his preparation of the material fail to demonstrate the cytoplasmic processes of the fibroblasts in the subendothelial layer? These questions and many others remained to be answered.

With heart diseases in humans on the increase any information

on the structure of the various tissues of the heart is of value. The following is a contribution to our incomplete knowledge of the endocardium of the rat heart. Whether it also applies to the human heart has yet to be established.

MATERIALS AND METHODS

Endocardium was taken from fifteen healthy adult albino rats, both males and females. The specimens were chosen from various regions of the atrial and the ventricular walls, from the valves, and from the medio-endothelial junctions of the adjoining major blood vessels.

Most of the material was fixed in 1% osmium tetroxide solution, buffered with acetate-Veronal buffer of Michaelis, which is the so-called Palade's Fixative. The pH of the fixative was adjusted to 7.3 - 7.5. The time for fixation was 1 - 2 hours. In some cases the specimens were fixed in buffered osmium - permanganate fixative according to Tahmisian (Pease 1964). Still others were fixed in a combined formaldehyde - glutaraldehyde fixative, followed by post fixation in 1% buffered osmium tetroxide. This fixation was introduced by Karnovsky (1965).

A graded series of diluted ethyl alcohol solutions were used for dehydration, starting with 50% ethyl alcohol, then 70%, 95% and twice into absolute alcohol. The tissue stayed in each bath for approximately 5 - 10 minutes. They were shaken gently every few minutes.

Most of the specimens were embedded in maraglas 655. This is an epoxy resin which was first introduced by Freeman and Spurlock (1962). Following dehydration, the tissues were kept in propylene oxide for 20-30 minutes, then placed in a 1:1 mixture of propylene oxide and maraglas epoxy medium for about 30 minutes. Following this the tissues were placed in cold maraglas epoxy medium for 15-20 hours,

to allow complete infiltration. Then the specimens were embedded in maraglas mixture in gelatine capsules and left to polymerize at 60°C for 36 - 48 hours.

Some specimens were embedded in epon 812 embedding medium, according to Luft. (Pease 1964).

A Cambridge ultramicrotome was used for sectioning the specimens. Glass knives were used.

Copper grids, 2½ mm in diameter of 150, 200, and 400 mesh with a supporting formvar film were used. In some cases no supporting film was used, instead the sections were taken and stored on bare 400 mesh grids.

Working with ultrathin sections, contrast is a difficulty. When the material consists of a substance made up of light atoms, having approximately the same density as the embedding medium and the supporting film, contrast is an intrinsic difficulty. Contrast depends largely on "background fog" that the photographic plate records. Staining for electron microscopy consists of adding heavy atoms to the specimen. When osmium tetroxide is the fixative used, the tissues are stained during fixation to a certain extent.

At the dawn of electron microscopy, when methacrylate was used as an embedding medium, phosphotungstic acid and uranyl acetate were the most commonly used stains. In 1958, Watson introduced a lead hydroxide stain, which was a great improvement over previous stains used. Most recently Reynold's (1963) lead citrate stain has become a commonly used stain for ultrathin sections for electron

microscopy. By using citrate as a chelating agent in sufficient excess to remove all of the lead present, it quite effectively prevents the lead from combining with carbonate. Potassium permanganate stain which was introduced by Lawn in 1960 (Pease 1964) has become a general stain for electron microscopy. These above mentioned stains were most commonly used during these investigations and in addition, Ruthenium red and Indium trichloride were also used for staining purposes.

For these investigations two electron microscopes were used, the JEM - 30 and Philips EM 200.

The quality of electron micrographs depend solely upon the optical properties of the microscope and the preparation technique employed, provided that optimum use is made of the microscope's photographic apparatus. In the electron microscope the image arises from scattered electrons. The quality of the image then depends upon the type of emulsion, the intensity of the radiating source, and the processing method used.

For these investigations 35 mm Kodak fine grain positive film was used for the negative material. Kodak Kodabromide 2, 3, 4 and 5 paper for prints. The processing comprises the following manipulations with the negatives and the papers:

1. Developing: Time and temperature according to the data in the formula. Kodak Dectol, Kodak D_{11} and D_{19} were most commonly used.
2. Stop Bath: A 3% solution of acetic acid, 15 - 30 seconds, Continuous agitation.

3. Fixing: Kodak acid fixer was used 5 - 10 minutes average time for negatives, 10 - 15 minutes for prints. After fixing, the chemicals were removed by carefully rinsing the films in a rapid flow of fresh water for 30 minutes, and the prints for at least one hour. The films were finally washed in photoflow and dried at room temperature. After being washed, the prints were bathed in flexogloss and dried on a Kindermann rotary stainless steel dryer.

For purposes of light microscopy several hearts were removed from adult healthy albino rats, and fixed in 10% formalin fixative, Carnoy's fixative or Zenker's fluid. These fixatives were made up according to standard procedures described for general microtomy. Following the fixation a routine method for dehydration according to fixative used was carried out. The embedding medium in all cases was paraffin. The tissues were sectioned with a Spencer "820" microtome. Serial sections of 6 - 8 microns in thickness were mounted on 25 x 75 mm microscope slides. They were stained with the following stains: Hematoxylin - eosin, Masson trichrome and Weigert's resorcein fuchsin elastic stain. The sections were mounted with synthetic medium and examined with the aid of a NF - microscope. EX - 135 30-Kodak panatomic X black and white films were used for photographic purposes.

OBSERVATIONS

Endocardium, which forms a complete lining for the atrial and ventricular cavities and covers all the structures that project into these cavities, is connected to the endomysium of the myocardium and is continuous with the intima of the blood vessels that leave the heart. Endocardium consists of two layers, a single layer of endothelial cells, and a subendothelial loose connective tissue layer extending to the myocardium. The endothelial cells lining the cavities of the heart thus are exposed to blood at the luminal side but separated from the myocardium by the subendothelial connective tissue layer, subendocardium, endocardium proper or subendothelial layer.

The Endothelium

The cells are polygonal squamous and rest upon a basement membrane. (Figs. 38, 42, 44). They are thickest in their nuclear region, where they bulge into the cavities. The nuclei are centrally located and usually oval in shape. The cytoplasm of the endothelial cells is most abundant at the periphery of the nuclei, but becomes greatly attenuated towards the cell junction. The nuclei have a thin rim of cytoplasm at both basal and apical portions.

The plasma membrane consists of two electron opaque layers separated by a light interspace. (Figs. 2, 5, 11, 26, 31). It measures 75 - 90 Å in thickness. Where deeply stained, the plasma membrane of the endothelial cells measures 120 Å or more. The plasma membrane does not show microvilli or other slender processes, except

at the cell junctions, but on both apical and basal surfaces the plasma membrane invaginates and detaches to form pinocytotic vesicles. (Figs. 1, 39.) These plasma membrane invaginations vary in shape and size. The largest measures up to 900 Å in diameter, but on the average their diameters range from 450 to 600 Å. After losing their attachment to the plasma membrane they are found free in the cytoplasm. (Fig. 29).

The endothelial cell nucleus is relatively large compared to the size of the cell. Examination of serial sections indicates that the nuclei often have an irregular saucer - like shape with a diameter of 8 - 9 microns and thickness of 1 - 2 microns. However, irregularity in shape is quite common and indented nuclei are found frequently. (Figs. 8, 9). The membrane surrounding the nucleus is double with a space between the two membranes of 200 - 400 Å. (Fig. 2). The nucleus has one or more nucleoli (Figs. 16, 18). The nucleolus appears as a dense irregular mass consisting of a tight aggregation of spherical particles of about 150 - 180 Å in diameter or slightly elongated bodies approximately 160 x 250 Å in size. There is no limiting membrane around the nucleolus. The bulk of the endothelial cell nucleus consists of a fine filamentous material and granules of macromolecular size. (Figs. 2, 3, 18, 31).

The cytoplasm of the endothelial cells contains the usual organelles. From examination of serial sections it is evident that these are relatively poorly developed. Mitochondria are few in number, usually small, slightly oval in shape with few cristae.

(Fig. 2, 3, 23, 29). A rough estimate indicates that there are approximately 8 - 10 mitochondria per endothelial cell. They show an outer membrane, approximately 60 - 80 Å thick and an inner membrane of similar thickness and character. The space between these two membranes measures 80 - 100 Å.

Golgi apparatus and endoplasmic reticulum are found in the cytoplasm of the endothelial cells. The Golgi apparatus is always in a paranuclear position. Centrosomes are often found in close association with the Golgi apparatus. (Figs. 16, 17).

In the endothelial cells the endoplasmic reticulum is poorly developed, but both granular and agranular endoplasmic reticulum are present. These appear as short tubular profiles. (Figs. 1, 2, 6,9,).

Free cytoplasmic ribosomes are few in number. The ribosome particles appear to be spherical bodies of about 150 Å in diameter, or slightly elongated particles of 150 - 250 Å in length. (Figs. 1, 3, 5).

Lysosomes and related microbodies are scarce but present. (Fig. 23). They appear as spherical granules. Their diameters range from 0.2 - 1.5 microns. These bodies are limited by a single well-defined membrane. They contain a fairly granulated substance which often appears as small round bodies, of 200 - 300 Å in diameter, close to the limiting membrane of the lysosome.

The bulk of the cytoplasm of the endothelial cells is often filled with micropinocytotic vesicles. (Fig. 29). As earlier described the vesicles are found attached to the plasma membrane at the apical

surface along the base and at the interfaces of the cell junctions. The micropinocytotic vesicles are round bodies limited by a single membrane about 40 - 50 Å in thickness. Their electron density is somewhat greater than that of the cytoplasmic matrix. (Fig. 39).

Occasionally, at the lumenal surface of the endothelial cells, clumps or vesicles of somewhat vesiculated material are found. (Figs. 30, 28). These are limited by a single membrane. They are also found at the base of the cells and throughout the subendothelial layer.

The endothelial cells rest upon a basement membrane. (Fig. 42, 44). This is often electron lucid closest to the plasma membrane, but becomes electron dense at a distance of 100 - 200 Å from the cell. The basement membrane is often replaced by a thicker basal lamina (Figs. 17, 18, 28, 30, 32). This basal lamina measuresup to several thousand angstroms in thickness. It seems that collagen and reticular fibrils run parallel at the base of the endothelial cells and these fibrils are the major components of this laminated basal layer.

The junctions between adjacent endothelial cells are found to be of great complexity and variability. They can be described as having an "S - shape". (Figs. 11, 39), a "wedge - shape" (Figs. 6, 18, 31, 32), or a "V - shape" (Figs. 3, 17). More complex junctions between adjacent endothelial cells can be found such as a "complex" consisting of overlapping, membranous folds and interdigitations. Such "junctional complexes" are illustrated in figures 1, 5, 25, and 26. In figure 5 two cytoplasmic processes are shown in a junctional complex

engaged at a 90° angle. The diameter of these processes measures approximately 1500 - 2000 Å. It is noticeable that the cell junction shown in figure 17 as V-shaped becomes S-shaped in figure 18 at a distance of 1500 - 1800 Å. Finally the so-called marginal fold junctions are present. These are classified as "open marginal fold junctions" and "closed marginal fold junctions". They are illustrated in figures 14 and 15 respectively. Desmosomes are not found at the endothelial cell junctions. The intercellular space usually measures 150 - 220 Å across.

Very little difference in fine structure is noticeable between the endothelium of the ventricles and that of the atria. The cells in the atrial endocardium are a little thicker than those in the ventricles, and the nuclei are more often indented in the atrial endothelial cells. Occasionally the cytoplasm of the atrial endothelial cells contains a few fibrils or microtubules. These are not found in the ventricular endothelial cells.

The Subendothelial Layer

The subendothelial layer of the endocardium underlying the simple epithelium is a loose connective tissue layer. It is thicker in the atrial than in the ventricular walls. (Figs. 53, - 56). In the ventricles it measures from less than a micron in thickness, where the myocardiac cells bulge out, to several microns in thickness where it adjoins the cardiac muscle associated tissue space. In the atrial walls the thinnest portion of the subendothelial layer measures over a micron, but usually it measures several microns. This layer

contains groups of components, such as cells, fibrous elements, capillaries and unmyelinated nerve endings embedded in an amorphous semisolid gel. (Figs. 1,4,6,9,12,21,43,52). The extracellular space and its components, thus bounded by the basement membranes of the endothelium and the myocardium, are found to extend in between the muscle cells to be finally continuous with that of the epicardium. The gaps in between the various myocardiac cells are of all sizes and shapes. No distinction can be drawn between the endocardium and the myocardial associated tissue space. It is in these wider areas of the subendothelial layer that the different components such as unmyelinated nerve endings, capillaries, Schwann cells, and fibroblasts are commonly located. This is so especially in the ventricular walls. However, nerve endings and fibroblasts are found close to the basement membrane of the endothelium. (Figs. 42, 43). Capillaries are present only in the deeper regions of the subendothelial layer. (Fig. 21). The subendothelial layer of the atria, which is somewhat thicker than in the ventricles, contains a slightly more electron-dense extracellular substance than that of the ventricles. Here the fibroblasts and Schwann cells run usually in a parallel pattern between the endothelium and the myocardium. Collagen fibrils are more abundant, but do not form dense fibers like those found in the elastic ring or in the valves.

In the subendothelial layer fibroblasts are the cells which are most commonly found. There they are embedded in the ground substance in a fairly irregular fashion. A rough estimate indicates

that the ratio of fibroblasts to endothelial cells in the ventricular walls is 1:2 but 2:1 in the atrial walls. The fibroblasts have large nuclei, ovoid in shape and often with a slight indentation on one side. The ultrastructure of the fibroblast nucleus is similar to that of the nucleus of the endothelial cells. (Fig. 32). The fibroblast nucleus is often surrounded by a thin rim of cytoplasm which may extend into the ground substance in long slender processes. (Figs. 6, 10, 16). At the margin of the cell where these processes arise, the cytoplasm is more abundant and contains numerous large vacuoles of irregular sizes and shapes. (Figs. 43 - 45). Dense inclusion bodies are found in association with these vacuoles. (Fig. 43). Examination of serial sections indicates that in these regions large pores are present in the plasma membrane through which the matrix of the vacuoles is extended into the extracellular ground substance. (Fig. 44). Collagen fibrils are found in close association with these regions.

Deeper within the cytoplasm, closer to the nucleus, a clear region the so-called centrosome is found surrounded by Golgi complex. (Figs. 40, 44, 45). Within the centrosome two small cylindrical bodies, the centrioles lie. Their approximate dimensions are 1500 $\mathring{A}$ in diameter and 5000 $\mathring{A}$ long. Each cylinder is made up of nine pairs of small tubules each about 200 $\mathring{A}$ in diameter forming a circle at the periphery.

The Golgi apparatus is well developed. Its lunar shaped cisternae complex is associated with small vesicles and larger vacuoles. (Fig 45).

The endoplasmic reticulum in the fibroblasts is well developed. It is often agranular in the region close to the Golgi complex (Fig. 45). The granular endoplasmic reticulum is found throughout the main portion of the cytoplasm and in the cytoplasmic processes that arise from the main cell body. (Figs. 1, 11). The endoplasmic reticulum is present as short tubular profiles, medium-sized cisternae and large cisternae, in association with vacuoles of various sizes and shapes which have already been described. (Figs. 43-45).

Mitochondria are few in number, usually small, oval in shape, with few cristae. (Fig. 45).

Lysosomes are present. (Fig. 40). These are spherical bodies, slightly smaller than mitochondria. They contain a finely granulated substance which is believed to contain a number of "digestive" enzymes. The matrix of the lysosomes is limited by a single well defined membrane 55-60 $\mathring{A}$ thick.

Unmyelinated nerve fibers are present in the subendothelial layer of both atrial and ventricular walls. (Figs. 6, 7, 12, 19, 20, 21, 22, 42, 43). The nerve fibers are bundled together within the cytoplasm of the Schwann cells. Single fibers are found enveloped by a thin rim of Schwann cell cytoplasm and its plasma membrane. The plasma membrane of the Schwann cell forms deep invaginations in which are embedded the individual neuraxons. (Figs. 19, 20). Narrow channels are defined by the apposition of portions of Schwann cell cytoplasm. The channels and membranes facing them constitute the mesaxon. The neuraxons, which are composed of the so-called

axoplasm, and the Schwann cell cytoplasm are separated by two unit membranes. Each unit membrane is approximately 75 - 90 Å thick with a space of 100 - 200 Å between them. One of these is the plasma membrane of the Schwann cell and the other is the so-called axomembrane. In ultrastructure, these two unit membranes are identical. They resemble the plasma membrane of the endothelial cells previously described.

The axoplasm matrix appears in the form of gel-like or amorphous ground substance in which formed elements such as mitochondria, neurofilaments, granules and synaptic vesicles are embedded.

The filaments which are believed to be a fibrous protein are approximately 100 Å thick. (Fig. 42). They are associated with dense granules. The filaments are not arranged in a regular pattern, but generally they are orientated longitudinally within the axoplasm.

The nerve fibers, which are found throughout the subendothelial layer, contain numerous synaptic vesicles. These have a diameter of 400 - 500 Å and a limiting membrane of 40 - 50 Å.

In the subendothelial layer the cytoplasm of the Schwann cells is relatively sparse in comparison with the axoplasm. Its cytoplasmic organelles such as the mitochondria and the endoplasmic reticulum do not show any characteristic difference from those of the endothelial cells or fibroblasts.

The Schwann cell nucleus is a large oval or round body. Slight indentations often occur where neuraxons are pressed against the nucleus.

The unmyelinated nerve fibers with their Schwann cell sheath

are surrounded by an electron-dense basement membrane material 350 - 450 Å thick.

Myelinated nerve fibers were not found in the subendothelial layer.

Capillaries are present in the deepest portions of the subendothelial layer only. (Figs. 21, 38). In cross sections two or more endothelial cells encircle the lumen of the capillary. The interface of the endothelial cells often contains a desmosome. Here the membrane is more dense than the rest of the plasma membrane, and measures approximately 200 - 250 Å in thickness. The portion of the plasma membrane exposed to the lumen of the capillary is often called the inner membrane of the endothelial cell, and the basal portion the outer membrane. Pinocytotic vesicles are numerous within the cytoplasm of the endothelial cells. They range in diameter from 400 to 600 Å. They may be free within the matrix, but more often they are found associated with the inner and the outer plasma membranes of the capillary endothelial cells. The cytoplasm contains the usual organelles. The endothelial cells vary in thickness from 0.4 to 1.2 microns. A well defined basement membrane, approximately 400 - 500 Å thick encircles the capillary.

The extracellular space of the subendothelial layer is fairly electron-lucid, especially in the ventricular walls. Collagen is found throughout the layer, as single unit fibrils or in bundles of several unit fibrils. These may run either parallel to the endothelium which most often occurs in the atrial walls or irregularly arranged

in the ground substance. The collagen fibrils are denser and more numerous in the endocardium of the atria than in the ventricles. The unit fibrils measure about 500 - 600 Å in diameter. They show a major periodicity of 600 - 700 Å. (Fig. 51).

Elastic clumps or lamellae are only present in the "subendothelial layer" at the aortic junction. (Figs. 49, 52, 58).

The Endo-Myocardial Junction

Electron microscopy studies on the structure of mammalian cardiac muscle were done by Porter et al. (1957), Sjostrand et al. (1960) and by many others. These investigations paid special attention to the junction between the endocardium and the myocardium.

Where the endocardium and the myocardium come in contact, the plasma membrane of the muscle fibers is associated with an extracellular basement membrane. Together these constitute the sarcolemma. The sarcolemma is about 400 - 650 Å thick. It consists of a unit membrane 60 - 80 Å in thickness and an outer basement membrane, which displaces an inner electron lucid layer about 100 Å thick and an outer moderately electron-dense layer. (Figs. 33, 41). In longitudinal sections the sarcolemma is often found to invaginate opposite each Z line. (Figs. 24). Thus between two Z lines the sarcolemma bulges out. There the mitochondria are present in large numbers. Sarcoplasmic reticulum is found close to the sarcolemma, but continuation between these is fairly uncommon. The transverse tubular system is not as prominent as in skeletal muscle and triads are rarely seen. The sarcoplasmic reticulum is found in association with well organized

arrangements of the mitochondria, and the tubules appear to be somewhat twisted or spiral in shape. (Figs. 36, 37, 41).

One of the most important contributions made by electron microscopic studies on the myocardium has been the elucidation of the boundaries of individual cells i.e. the intercalated discs.

This investigation paid attention only to the structural details of the intercalated discs adjacent to the endocardium.

In longitudinal sections the intercalated discs are seen as irregular zigzags between the plasma membrane surfaces. (Figs. 33, 34, 35). The discs are modified cell boundaries of adjacent myocardial cells. The investigation revealed that these modified cell surfaces exhibit several different structural forms. Two cell membranes of medium density with an interposed intercellular space of variable width may be seen. These may occur in a plane either parallel to or at right angles to the long axes of the myofibrils. Where the distance between the two adjacent unit membranes is nil, the portions of the intercalated disc display the so-called tight junction (zonula occludens). This type is common where the intercalated discs are exposed to the subendothelial layer. (Fig. 33). Where the distance measures 150 - 250 Å between unit membranes the junctions are called intermediary junctions (zonula adhaerens). This is the most common type. Desmosomal-like bodies are also found in the intercalated discs, but well developed desmosomes are scarce.

The unit membranes of the sarcolemma and the intercalated discs are continuous. However, the basement membrane of one sarcolemma

is continuous with that of the next sarcolemma passing over the intercalated disc. (Figs. 33, 35).

As mentioned earlier where the cardiac muscle associated tissue spaces merge into the subendocardium no definite boundaries can be drawn.

The Valves

The ultrastructure of the heart valves differs considerably from the rest of the endocardium. Two layers of endothelium are separated by a relatively dense connective tissue. (Figs. 46, 47, 48, 57, 59).

The endothelial cells are extremely flat. The cytoplasm contains medium sized vesicles and numerous dense particles. Mitochondria, endoplasmic reticulum and ribosomes are scattered throughout the cytoplasm. The nucleus is flat and irregular in shape. It contains dense chromatin granules and one or more nucleoli.

The subendothelial layer of a valve consists of relatively dense connective tissue. The fibroblasts are extremely flat and are arranged in somewhat irregular membranous plates, separated by substantial layers of collagen fibrils. The fibrils are arranged in bundles, which often are oriented at right angles to one another. The fibers and the fibroblasts are embedded in an amorphous ground substance. Neither capillaries nor unmyelinated nerve endings were found in the subendothelial layer of a valve. Elastic material is not present.

The Aortic Fibrous Ring

The aorta and the pulmonary artery arise from the left and the right ventricles respectively. At its point of origin each is surrounded by a fibrous ring. These fibrous structures, together with the membranous part of the interventricular septum, also provide a means for the insertion of the free ends of the cardiac musculature. In this study only brief attention was paid to the structural details of the junction between the aorta and the ventricular wall. (Figs. 49, 50, 57).

The endothelial cells at this junction are flat. The cytoplasm contains dense particles and medium-sized vacuoles. The nuclei of the endothelial cells are often found with numerous indentations.

At the junction where the subendothelial layer of the endocardium merges with the aorta no myocardium is present. Here the subendothelial layer is thicker than it is elsewhere, and it blends with the media of the aorta. It is composed of collagen and elastic fibers embedded in an amorphous ground substance (Figs. 49, 50, 57). Few fibroblasts are found scattered irregularly among the fibers. The collagen fibers are mainly arranged in bundles which run in planes parallel to the endothelial cells and to the elastic laminae, whereas the elastin is mainly deposited as membranes, plates or clumps of irregular shapes and sizes. The elastic lamina appears to be composed of a dense homogenous macromolecular complex. These formed elements are embedded in an amorphous ground substance.

DISCUSSION

The Endothelium

The endothelium in adult rats is a thin layer. It is composed of thin squamous cells. These are joined together at their interfaces, which often are characterized by overlapping, membranous folds and interdigitations. The endocardial endothelium in adult rats is a complete layer and there are no deficiences found in it. Thus it differs from that of tree frogs. (Stehbeus et al. 1965). In the endothelial cells the cytoplasmic organelles are poorly developed. These were never found closely in association with the plasma membrane. However, numerous pinocytotic vesicles are found attached to the plasma membrane and free in the cytoplasm. This indicates the cells function with regard to active transport of fluid across the endothelium. Descriptions of pinocytotic activity by Palade (1953) and Fawcett (1956) in the endothelia of capillaries and arteries, by Buck (1958) in endothelium of large arteries and by Leeson et al. (1965) in the endothelium lining the cavernosus tissue in adult rat penis, indicate similarity to that of endocardial endothelium. However, these authors did not give a satisfactory explanation of the work involved in the pinocytotic activity of the endothelium. These studies showed that the plasma membrane of both the luminal and the basal surfaces of the endothelial cells invaginates forming small vesicles of about 600 - 700 $\mathring{A}$ in diameter. These, and the free vesicles in the cytoplasm of the cell obviously indicate transfer of fluid across the cells. Whether the direction of transfer is one way or two ways across the cell

is not possible to determine at this stage. It is assumed that the major route of transport is from the luminal border of the cell across to the base. Thus at the base the pinocytotic vesicle's content is released into the subendothelial layer. Only a limited explanation will be given here, regarding the pinocytotic activity in endothelial endocardium of adult albino rats.

The apical portions of the endothelial cells are exposed to the circulating blood. The basal portion of the endothelial cells is in a fixed position covering the subendothelial layer. Therefore, it is reasonable to consider the possibility in potential difference between the luminal plasma membrane and the basal plasma membrane. When pinocytotic vesicles are formed, a breakdown and reformation of the plasma membrane occurs. According to Robertson (1959) and several others the plasma membrane is a triple layered complex. There are two sheets of protein molecules separated by a bimolecular leaflet of lipid. These observations indicate that the limiting membrane surrounding the pinocytotic vesicles is 30 - 40 $\overset{o}{A}$ thick. This will correspond to half the thickness of the plasma membrane. When the pinocytotic vesicles are released into the cytoplasm reformation of the plasma membrane is essential. How this occurs is not possible to state. It might very well be that unbound lipid and protein molecules present in the cytoplasmic matrix are being used for purpose of reformation on the plasma membrane. A formed pinocytotic vesicle is a spherical body. Its diameter varies from 400 - 600 $\overset{o}{A}$. The limiting membrane is a sandwiched layer of

protein-lipid molecules orginated from the plasma membrane. The pinocytotic vesicles which are being formed and pinched off from the lumenal portion of the plasma membrane are thus charged with regard to the basal portion of the cell membrane. The pinocytotic vesicles are thus carried towards the base where discharge occurs. Following this an adhesion between the limiting membrane and the plasma membrane takes place and rearrangement of the lipid protein molecules opens up an entrance through which the contents of the vesicles are extruded into the subendothelial layer.

An outstanding feature of the endocardial endothelium is the boundaries between adjacent cells which are characterized by overlapping and membranous folds or large interdigitations. Candiollo (1963) describes overlapping of two contiguous cells, but he does not mention the possibility of membranous folds or interdigitations. Desmosomes have been reported to be present in endothelial junctions of capillaries and arteries Fawcett et al. (1962). However, these observations did not reveal desmosomes, even in the largest junctional complexes. In the junctional complexes the intercellular gap measures at least 100 $\mathring{A}$. The adjacent plasma membranes are never found to fuse. Wider spaces within the junctional complexes where pinocytotic vesicles are attached to the plasma membrane are found. These facts indicate that the junctions between adjacent endothelial cells are flexible. Thus the variations in the junctional complexes between adjacent cells is due to the movements of the heart, and the cells' ability to increase and decrease their surfaces.

As mentioned earlier the basement membrane upon which the endothelial cells rests is often replaced by a thicker basal lamina. The basal lamina can measure up to several thousand angstroms in thickness. It is a network of collagen and reticular fibrils randomly arranged in laminated layers at the base of the endothelial cells. This obviously will strengthen the endocardium and its elasticity will be kept within certain limits.

The Subendothelial Layer

The ultrastructure of the subendothelial layer in adult rat endocardium has not been described to any extent in the literature to date.

Candiollo's (1963) electron microscopy investigations did not extend to the various regions of the endocardium, but were limited to that of the papillary muscle only. His observation on the subendothelial layer is incomplete. He did not report on nerve endings and associated structures, nor on capillaries in the deeper regions. However, he believes that the subendothelial layer is "rich in elastic clumps". My investigations found no trace of such structures, except at the aortic junction. It is probable that Candiollo's preparations of the specimens failed to demonstrate properly nerve fibers, cytoplasmic processes of fibroblasts, and pinched off material from the endothelial cells. It therefore might very well be that these components of the subendothelial layer are classified by Candiollo as elastic clumps.

Capillaries are only present in the deepest regions of the subendothelial layer. According to the classification of Bennet et al.

(1959) they belong in type A-1-a which indicates that there is a continuous boundary membrane, no fenestrations or pores in the endothelial cytoplasm and an incomplete pericapillary cellular investment.

The observation of unmyelinated nerve fibers throughout the entire endocardium in adult rat is of significance. Figures 19, 20, 21 and 22 display nerve fibers in the deeper portions of the endocardium. Most of them contain numerous synaptic vesicles in their axoplasm. This will indicate the possibility of a neuromuscular junction in a close vicinity to the locations displayed. Figures 42 and 43 show a bundle of nerve fibers whose distance from the endothelial cells is less than 5000 $\overset{o}{A}$. Schwann cell nuclei are present in the deeper portions of the endocardium.

The subendothelial layer is a loose connective tissue. Collagen unit fibrils and small bundles of unit fibrils are scattered throughout the layer. Fibroblasts are numerous.

According to Revel et al. (1963), Yardley et al. (1960) and many other reporters on collagen synthesis, it is evident that fibroblasts are involved in collagen synthesis. However, to date no satisfactory report has been given on the question whether the collagen unit fibrils are formed outside of the fibroblasts or within the cells.

In many fibroblasts which are found within the subendothelial layer of the endocardium the profiles of cisternae form large anastomosing network of channels. This network, the endoplasmic

reticulum, has numerous closely packed ribosomes covering its exterior surface. At the pole of the fibroblast nucleus, where the cytoplasm is more abundant than elsewhere, the cisternae of the endoplasmic reticulum are connected to the Golgi complex by means of intracellular canaliculi. These electron microscopy studies indicate a formation of large irregular vacuoles in the region of Golgi complex and throughout the cytoplasm. Dense inclusion bodies are often found in association with these vacuoles. This is best seen in figures 43 and 44. In the regions where the vacuoles are exposed to the plasma membrane large pores are formed. The contents of the vacuoles are exposed to the extracellular substance and probably excreted. The examination of serial sections showed collagen unit fibrils outside the vacuolar pores and accasionally within the matrix of an "open" vacuole. Collagen unit fibrils were never found within "closed" vacuoles.

This will indicate that a "precipitate" of tropocollagen, which is undoubtedly present in the protein contents of the vacuoles, occur when the vacuoles discharge their contents into the extracellular space. This might be due to a difference in the pH between the matrix of the vacuoles and the extracellular substance.

Fine structure of the aorta in rats, was revealed by Pease et al. (1960), Keech (1960) and several others. However, these authors did not consider the fine structure of the aortic ring.

In the aortic walls the elastic laminae are flat fenestrated membranes which run parallel to the endothelium. Between the internal

elastic lamina and the endothelium is a thin layer of connective tissue. This layer is rich in collagen fibrils. Processes from the endothelial aspect of the lamina extend into the layer.

In the aortic ring the elastic laminae become masses of irregular plates and clumps. The main bulk of the aortic ring consists of dense connective tissue with irregularly arranged collagen bundles, fibroblasts and short elastic laminae embedded in an amorphous ground substance. This ring serves as a shock absorber between the aorta and the heart. The fibrous ring merges with the endocardium without any unusual structural characteristics. Where the fibrous ring meets the myocardium, elastic plates are no longer found. The collagen bundles become less numerous and the subendothelial tissue becomes the usual subendocardiac pattern (Fig. 52). This still is a further evidence, that the entire endocardium is free of elastic clumps or plates.

The Endo-Myocardial Junction

Where the endocardium and the myocardium come in contact, the marginal boundary is the basement membrane of the myocardium or the sarcolemma. Direct communications between the sarcolemma and the sarcoplasmic reticulum are extremely uncommon. However, in many areas close contact between segments of the sarcoplasmic reticulum and the unit membrane are found. As indicated in report of Stenger et al. (1961) these points of contact are most common at the Z line level and in contracted fibers are associated with infoldings of the sarcolemma at the Z line. Pinocytotic vesicles are found at the surface membranes.

This suggests active exchange of fluid between the myocardium and the subendothelial layer, for the purpose of nourishing the myocardiac cells.

The ultrastructure of the intercalated disc has been exhaustively analyzed by Sjostrand et al. (1960). These investigations paid attention only to that portion of the disc which is exposed to the endocardium. It is of interest to find that the basement membrane does not extend into the disc interspace. At most, the basement membrane forms a wedge between cells at the origin of an intercalated disc, without any significant extension into its interspace.

Variations of Endocardium

It is difficult to give any specific measurement of the varying thickness of the endocardium in adult rats. In the ventricles the endocardium cells are often extremely thin. The cytoplasm is often less than a micron in thickness. The subendothelial layer can be of similar thickness. In the regions where it merges with the cardiac muscle associated tissue space, a considerable thickening occurs.

On the contrary, the subendothelial layer of the ventricular endocardium measures several microns in width. Electron lucid, structureless extracellular connective tissue material is its main component. However, more electron dense basal laminae occur at the base of the endothelial cells.

The thickness of the atrial endocardium is considerably greater than that of the ventricles. However, the myocardium can be found at a distance of only a few microns from the lumen. The extracellular

matrix is here somewhat more electron dense than that of the ventricles. The basal lamina is not so distinctive as in the ventricular endocardium. Here the Schwann cell nuclei are found located in parallel position to the endothelium; whereas in the ventricular subendothelial layer they are only present in the regions where it merges with the cardiac muscle associated tissue space.

SUMMARY

1. The endothelial endocardium in adult rats is a complete layer or membrane. The numerous pinocytotic vesicles indicate their involvement in pinocytotic activity. The cell junctions are flexible. It is assumed that they are changing continuously due to the movement of the heart.

2. The basement membrane of the endothelial cells is replaced by a thicker laminated basal lamina which is composed of subendothelial fibrillar components. This will increase the strength of the endocardium and limit the elasticity of the organ.

3. Nerve endings are present in the endocardium in both the atrial and the ventricular walls. The numerous synaptic vesicles found in the axoplasm of the nerve fibers in close association to the myocardium is a strong evidence that they are receptor endings to the muscle cells.

4. Elastic clumps or plates are not found in the endocardium. Those are present only in the aortic ring but they are absent in the surrounding endocardium and elsewhere.

REFERENCES

BAKER, J. R. : 1963 Cytological Technique. Methuen Co. Ltd. London, England.

BARGMANN, W. : 1958 Uber du Structure der Blutkapilbren Dtsch. med. Wschr. 83, 1704.

BATTIG, C. G. and LOW, F.N. : 1961 The ultrastructure of human cardiac muscle and its associated tissue space. Am. J. Anat. 108, 2, 199.

BUCK, R. C. : 1958 The fine structure of endothelium of large arteries. J. Biophys., Biochem,, Cytol. 4, 187.

BENNET, H. S.: 1956 The concepts of membrane flow and membrane vesiculation as mechanisms of active transport and ion pumping. J. Biophys., Biochem., Cytol. 2, 99.

BENNET, H. S., LUFT, J. H. and HAMTON, J. D. : 1959 Morphological classification of vertebrate blood capillaries. Amer. J. Physio. 196, 381.

BRAIN, E. B. and CATE, A. R. T. : _____ Techniques in Photomicrography D. Van Norstrand Co., Princeton, New Jersey, U.S.A.

CANDIOLLO, L.: 1963 Fine structure of endocardial endothelium. Z. Zellforschung 61, 486.

DeROBERTIS, E., NOWINSKI, W. W. and SAEZ, F. A. : 1965 Cell Biology W. B. Saunders Co. Phildelphia and London 1965.

ELSON, D. : 1964 The ribosome, factory of the living cell, Discovery 25, 7, 24.

FARGUHAR, M. G. and PALADE, G. : 1963 Junctional complex in various epithelia. J. Cell. Bio. 17, 2, 375.

FAWCETT, D. W.:1956 Observations on the submicroscopic structure of small arteries, arterioles and capillaries. Anat. Rec. 124, 401.

FAWCETT, D. W.:1959 The fine structure of capillaries, arterioles and small arteries, in the microcirculation, Urbana, University of Illinois Press. 1.

GROSS, J. : 1965 Collagen. In readings from Scientific American p. 187, W. H. Freeman and Co. San Francisco, London.

HOLMES, R. L. : 1957 Structures in the atrial endocardium of the dog which stain with methylen blue and the effects of unilateral vagotomy. J. of Anat. 91, 2, 259.

HUXLEY, H. E. : 1961 Contractile structure of cardiac and skeletal muscle. Circulation, 24, 327.

JARCO, S. : 1958 Description of the endocardium by Bouillaud (1835) and Bichat (1800). Am. J. Card., 1, 767.

JAMIESON, J. D.: and PALADE, G.E. : 1964 Specific Granules in atrial muscle cells, J. Cell. Bio., 23, 1, 151.

KARNOVSKY, M. J. 1965 A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy, J. Cell. Bio., 27, 2, 137.

KEECH, M. K. : 1960 Electron microscopy study of the lathyrilic rat aorta., J. Biophy., Biochem, Cytol., 7, 3, 539.

KEECH, M. K. : 1960 Electron microscopy study of normal rat aorta. J. Biophy. Biochem., Cyto., 7, 3, 533.

LEESON, T. S. and LEESON, C. R. : 1965 The fine structure of the cavernosus tissue in the adult rat penis. Investigate Urology, 3, 2, 114.

MAYERSON, S. H.: 1962 Exchange of material across capillary wall, In blood vessels and lymphatics. Academic press, New York and London.

MORE, D. H. and RUSKA, H. : 1957 The fine structure of capillaries and small arteries. J. Biophy. Biochem, Cytol. 3, 457.

PALADE, G. E. : 1953 Fine Structure of blood capillaries, J. Appl. Phys., 24, 1424.

PALADE, G. E. : 1961 Blood capillaries of the heart and other organs. Circulation 24, 368.

PEASE, D. C. : 1964 Histological Technique for Electron Microscopy Second Edition. Academic press New York, London.

PEASE, D. C. and PAUL, W. J. : 1960 Electron Microscopy of elastic arteries, the thoracic aorta of the rat. J. Ultras. Research.,3, 469.

PORTER, K.R. and BONNEVILLE, M. A. : 1964 An Introduction to the fine structure of Cells and Tissue. Lea et Febiger, Philadelphia, U.S.A.

REYNOLDS, E. S. : 1963, The use of lead citrate as high pH as an electron-opaque stain in electron microscopy. J. Cell Bio., 20, 2, 350.

REVEL, J. P. and HAY, E. D. : 1963 An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. Z. Zellforch, 61, 110.

ROBERTSON, J. D. :1959 Biochem. Soc. Symp., 16, 3.

SIMPSON, F. O. And ORTELIS, S. J. : 1962 The fine structure of sheep myocardial cells, sarcolemmal invaginations and transverse tubular system. J. Cell Bio., 12, 1, 91.

SJOSTRAND, F. A. and ANDERSSON-CEDERGREEN, E. : 1960 Intercalated discs of heart muscle. In: Structure and Function of Muscle G. H. Burne, Ed. Academic Press, New York, 1, 421.

SMIRNOW, A. : 1895 Uber die sensiblen Nervendigungen im Herzen bei Amphibien und Saugetieren. Anat. Anz., 10, 737.

STEHBEUS, W. E. and MEYER, E. : 1965 Ultrastructure of endothelium of the frog heart. J. Anat. Lond., 99, 1127.

WOOLLARD, H. H. : 1926 The innervation of the heart. J. Anat. Lond. 60, 345.

ZAKI, S. A. and LANNIGAN, R. ; 1965 The ultrastructure of human auricle, J. Anat., 99, 10, 930.

ILLUSTRATIONS

Fig. 1. Electron micrograph from the muscular interventricular septum of the left ventricle demonstrating junctional complex (overlapping, membranous folds and interdigitation) between two adjacent endothelial cells.

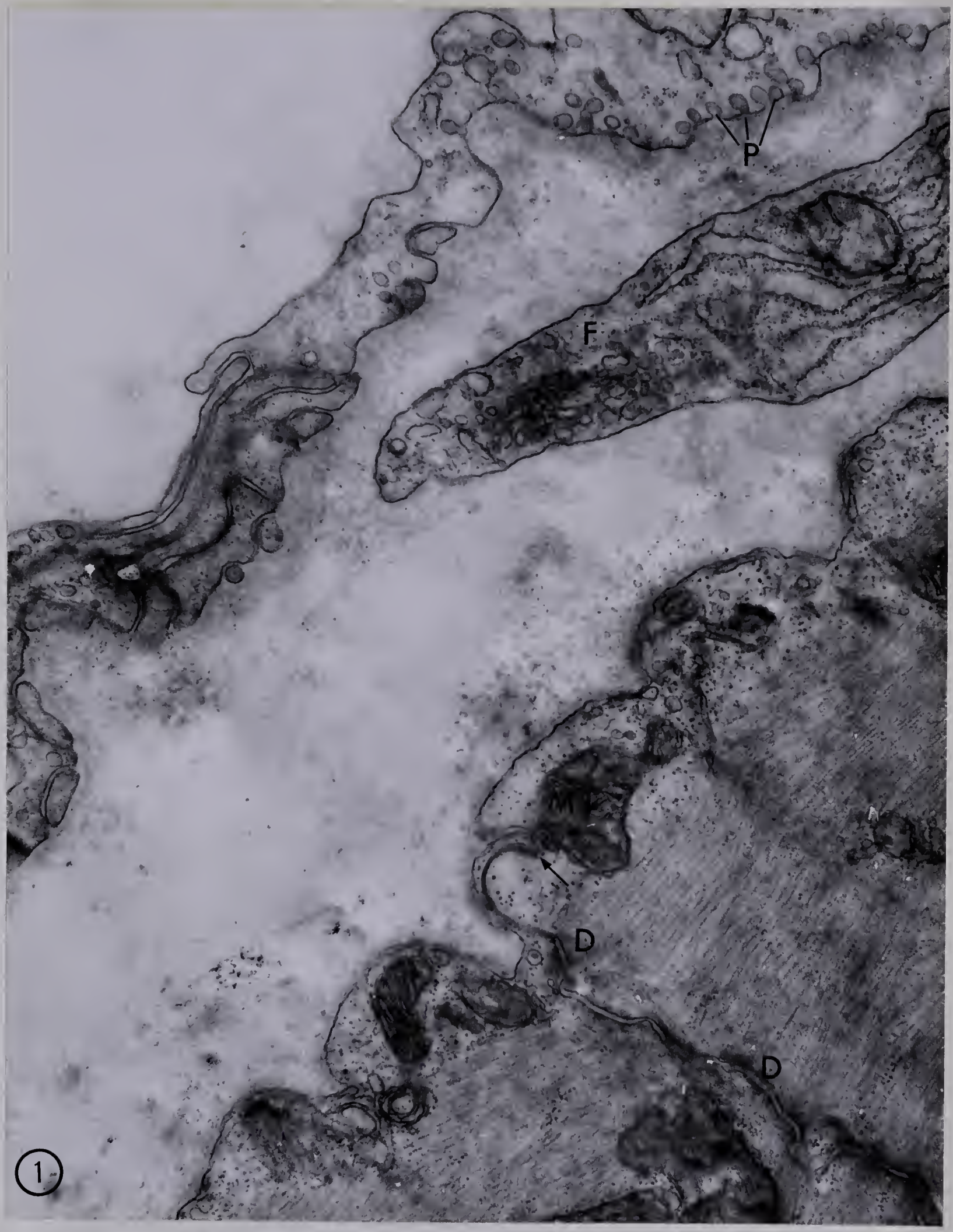
D: Desmosome like bodies in the intercalated disc

F: Fibroblast

MI: Mitochondrion

P: Pinocytotic vesicles

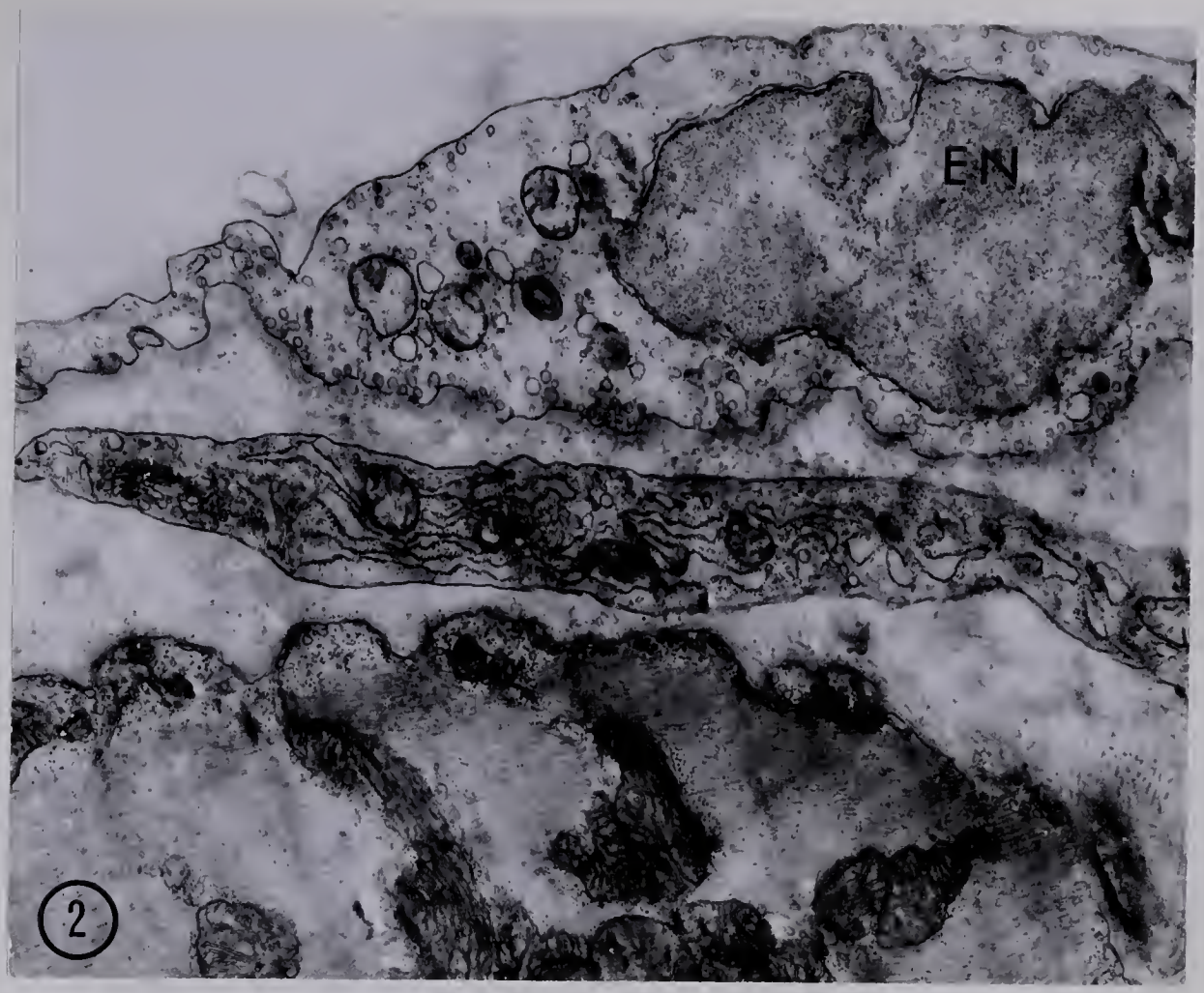
Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 38000



1

Fig. 2. Electron micrograph from a region near that displayed in Fig. 1. The endothelium (EN) is thickest at the nuclear region of the endothelial cell. A cytoplasmic process of a fibroblast runs parallel to the endothelium and the myocardium embedded in the extracellular connective tissue of the subendothelial layer.
Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 18500

Fig. 3. Electron micrograph from the lateral wall of the left ventricle. V-shaped cell junction between two adjacent endothelial cells are opposite to an extension of the subendothelial layer extending in between the muscle cells A deep indentation of the sarcolemma (arrow) is filled with basement membrane material.
Osmium tetroxide fixative, maraglas embedding and lead citrate staining. X 24000


EN
2

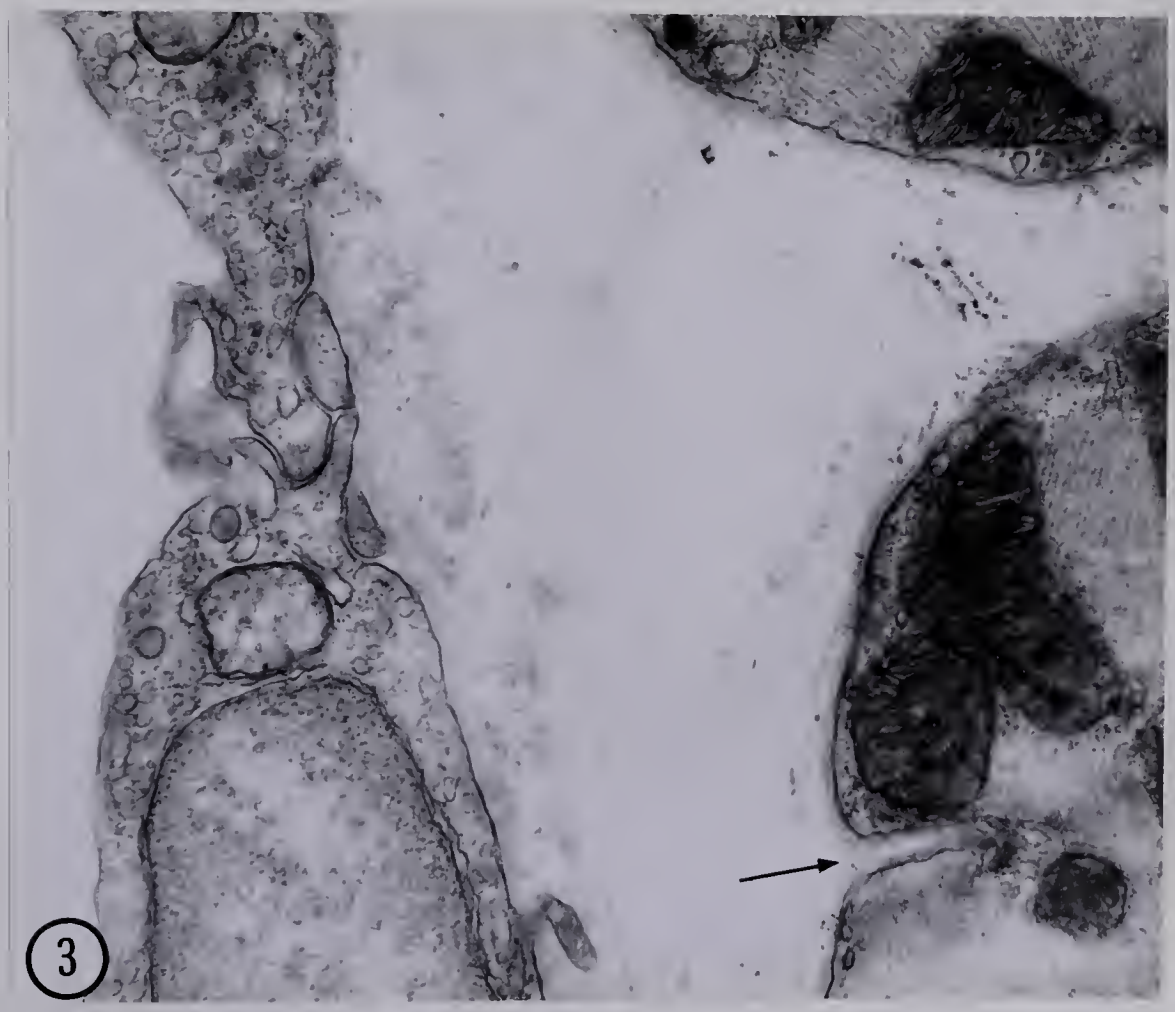

3

Fig. 4. Electron micrograph from the lateral wall of the right ventricle. The fibroblast (F) nucleus is somewhat triangular in shape and cytoplasmic processes might arise from each angle extending between the endothelial cells and myocardium and into the extracellular space of the myocardium.

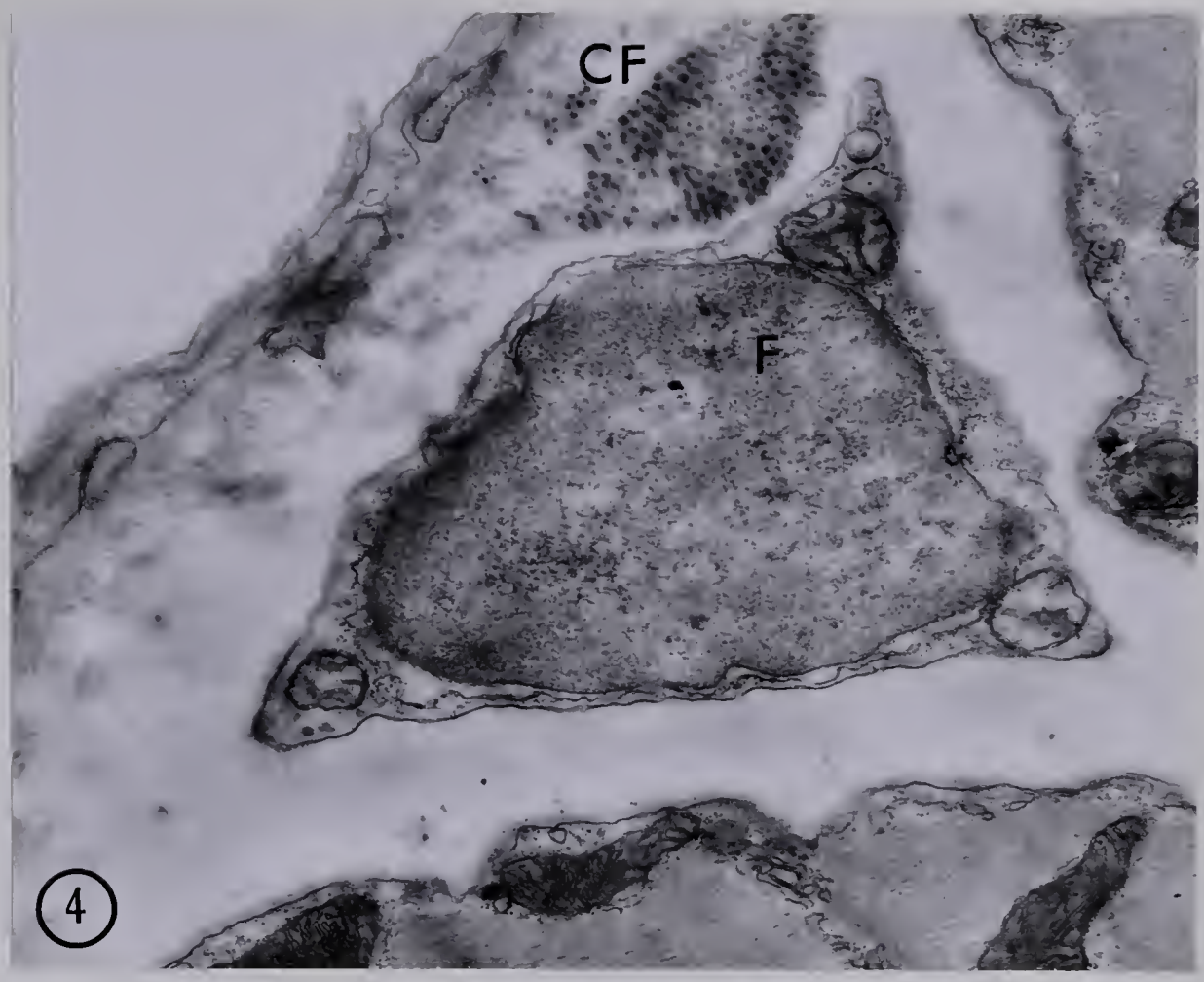
CF: Collagen fibrils.

Osmium tetroxide fixation, gluteraldehyde fixation and lead citrate staining. X 24000.

Fig. 5. Electron micrograph from the lateral wall of the right ventricle demonstrating a junctional complex between two adjacent endothelial cells. Two villi, one longitudinal cut, the other transverse (arrow) are making a right angle position towards each other.

MC: Myocardium

Glutaraldehyde - Osmium fixation, maraglas embedding, lead citrate and uranyl acetate staining. X 30.000.


CF
F
4

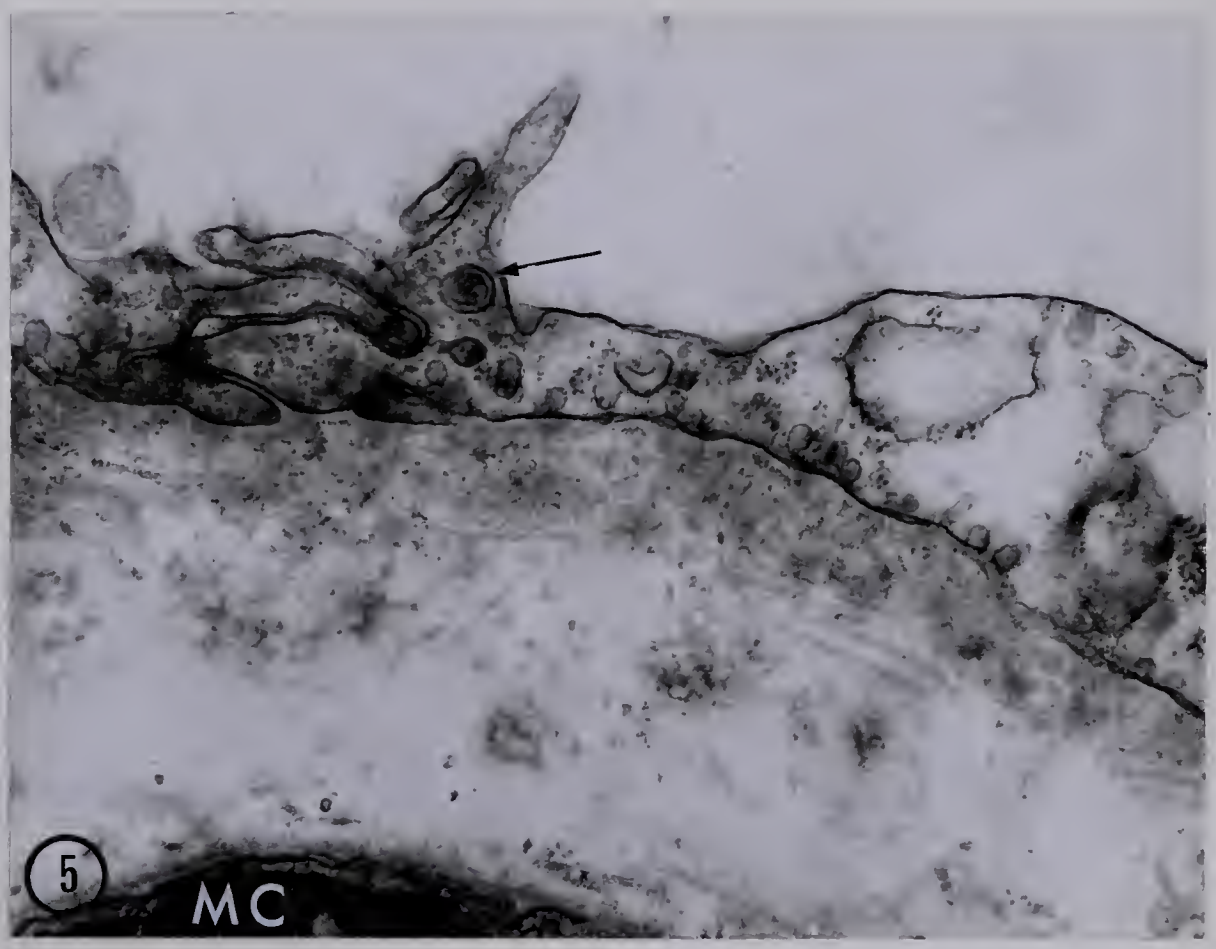

5
MC

Fig. 6. Electron micrograph from the superior anterior wall of the right atrium. A "wedge-shaped" junction between two adjacent endothelial cells is displayed.

GL: Glycogen particles

SC: Schwann cell

Glutaraldehyde - Osmium fixation, maraglas embedding potassium permanganate and lead citrate staining. X 20000.

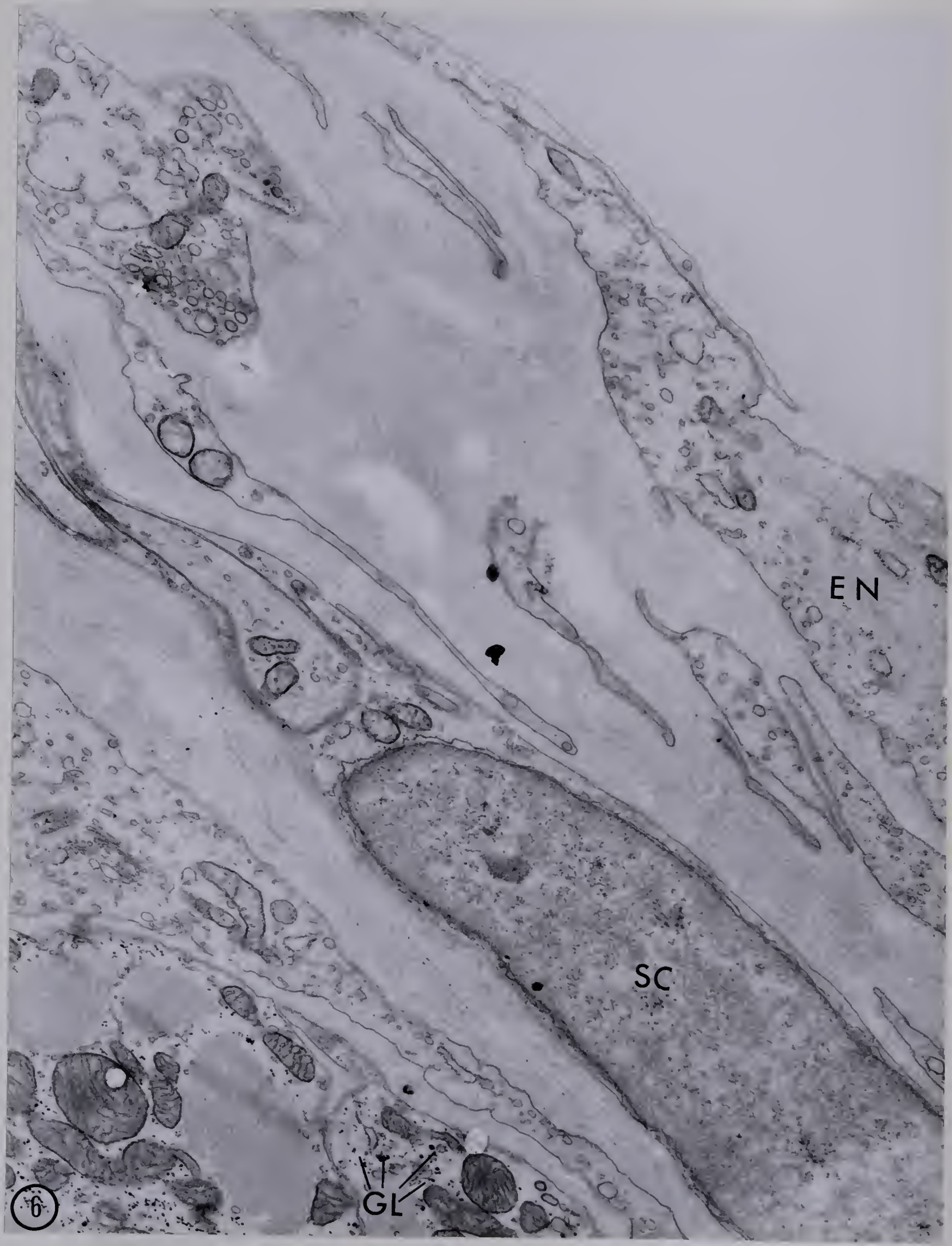

EN
SC
GL
6

Fig. 7. Electron micrograph demonstrating a portion of an unmyelinated nerve which is shown in fig. 6. Vesicles in the cytoplasm of the nerve fiber are being connected by fine filaments (arrow).

MI: Mitochondrion

X 40.000.

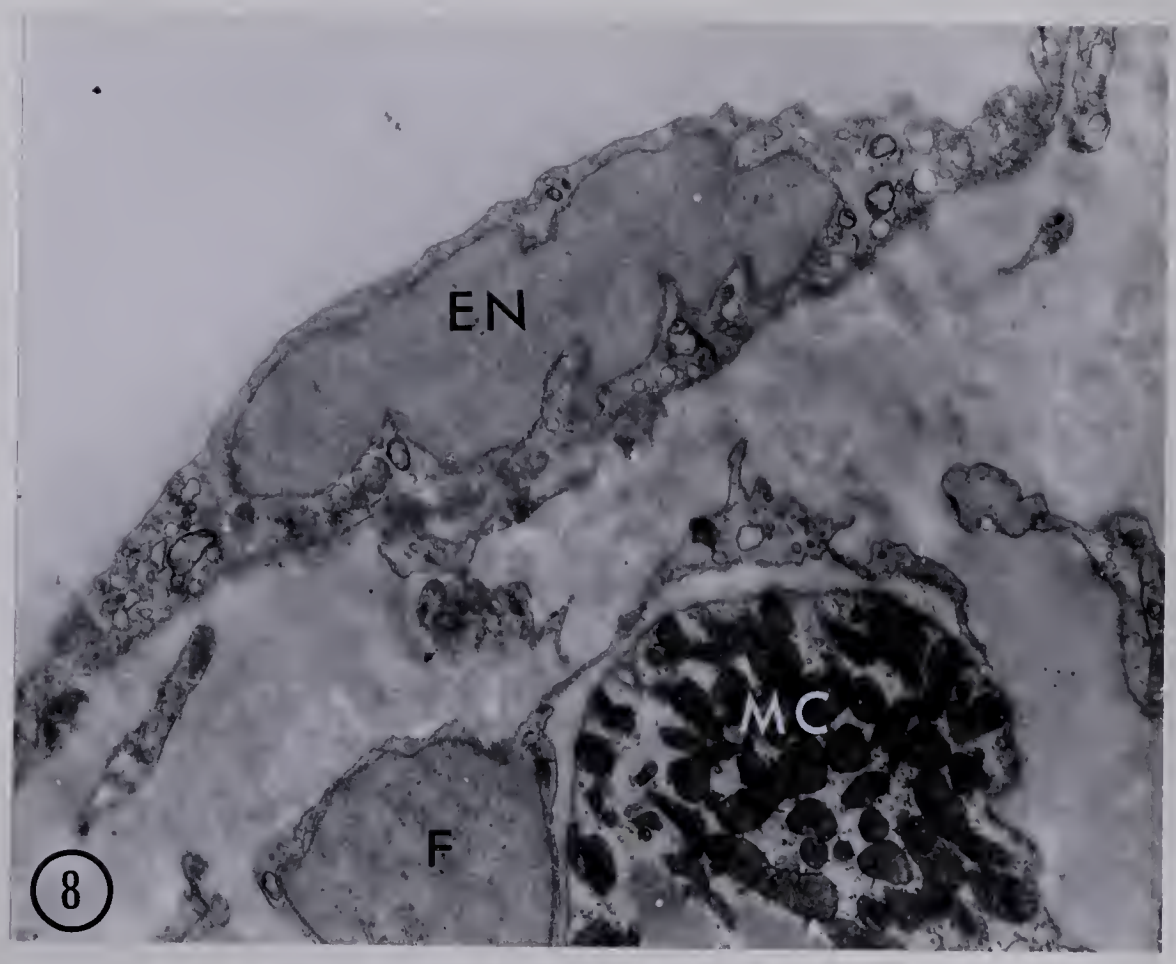
Fig. 8. Electron micrograph demonstrating an indented nucleus of an endothelial (EN) cell from a region close to that displayed in fig. 6.

F: Fibroblast

MC: Myocardium

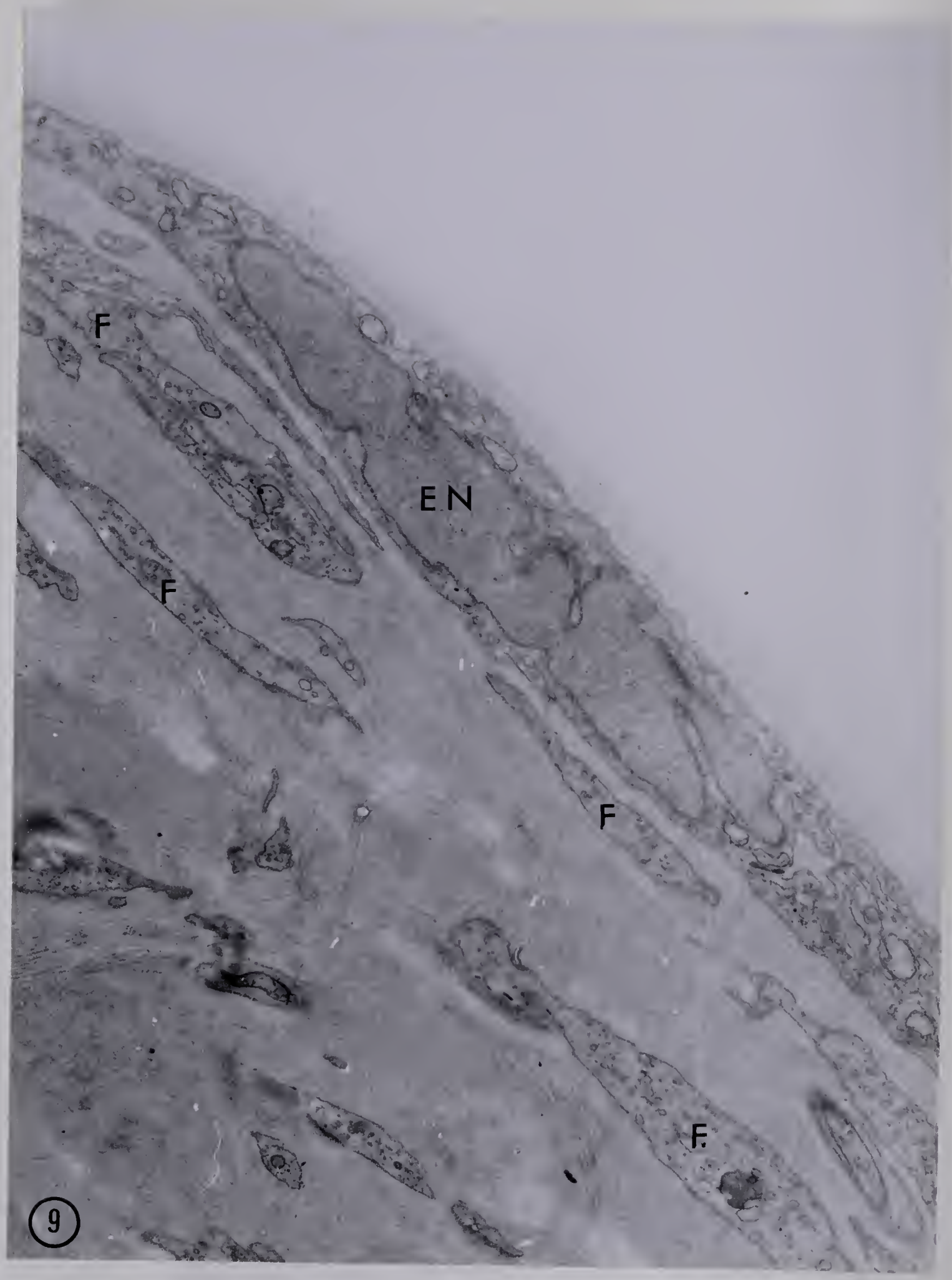
Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 8000.

7



8

Fig. 9. Electron micrograph from the inferior posterior wall of the right atrium demonstrating indented endothelial nucleus (EN) and numerous cytoplasmic processes of fibroblasts (F). Glutaraldehyde - Osmium fixation, maraglas embedding, lead citrate and uranyl acetate staining. X 13000.



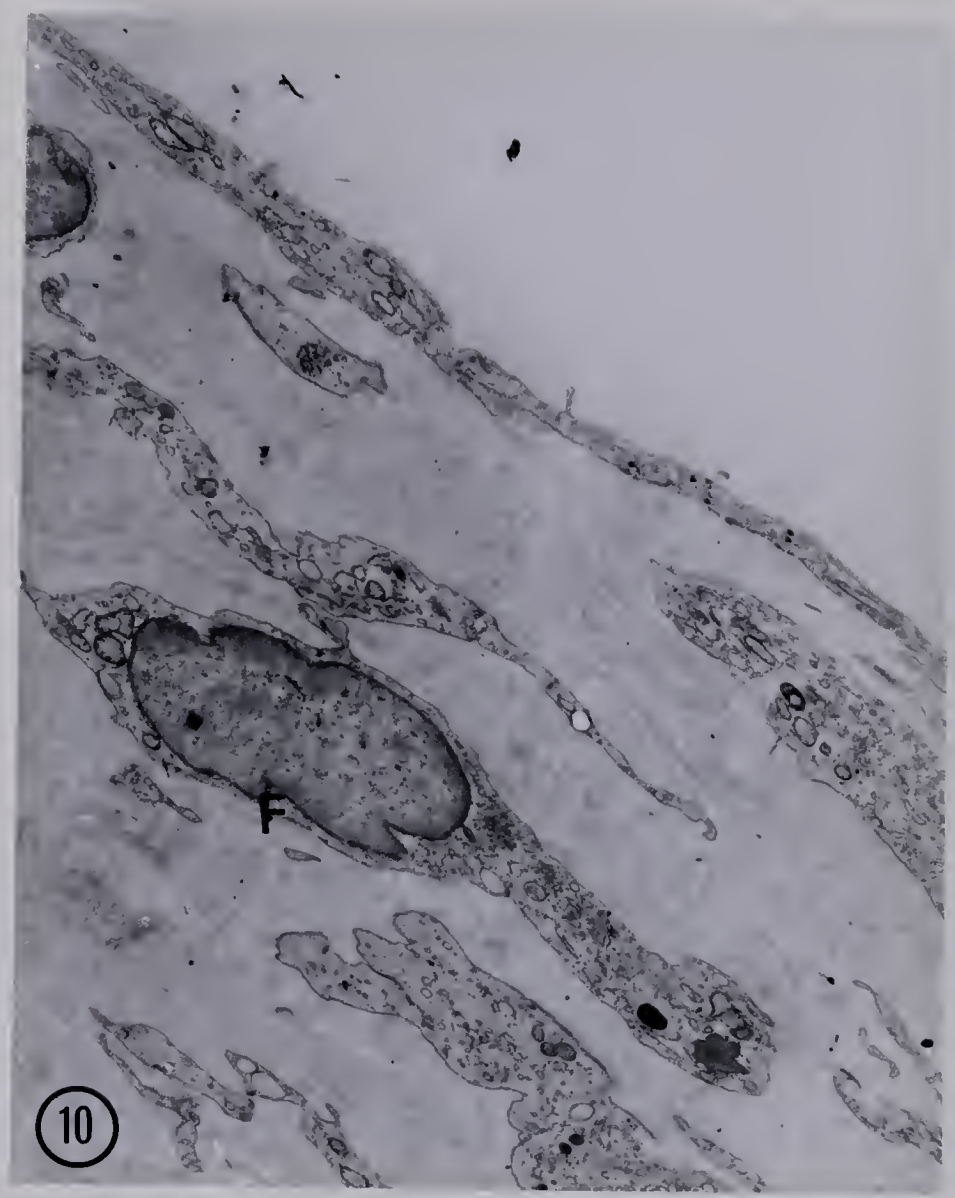
9

Fig. 10. Electron micrograph from a region near that shown in fig. 9.

F: Fibroblast

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 5500.

Fig. 11. Electron micrograph from the same section as shown in fig. 10, demonstrating an "S-shaped" junction between two adjacent endothelial cells (arrows), and a portion of a fibroblast's cytoplasmic process. X 18000.


F
10

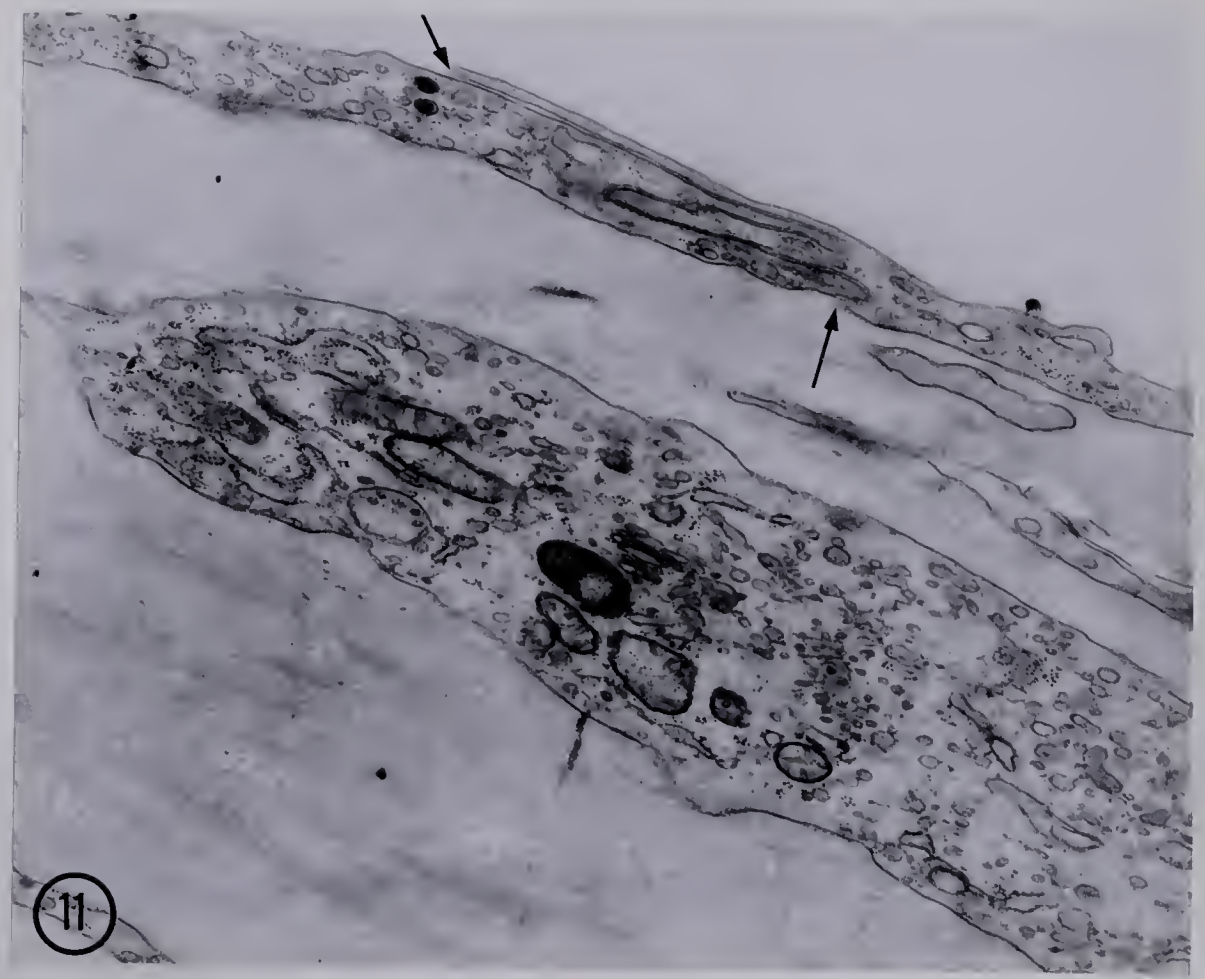

11

Fig. 12. Electron micrograph from the inferior anterior wall of the right atrium.

CF: Collagen fibrils

EN: Endothelium

F: Fibroblast

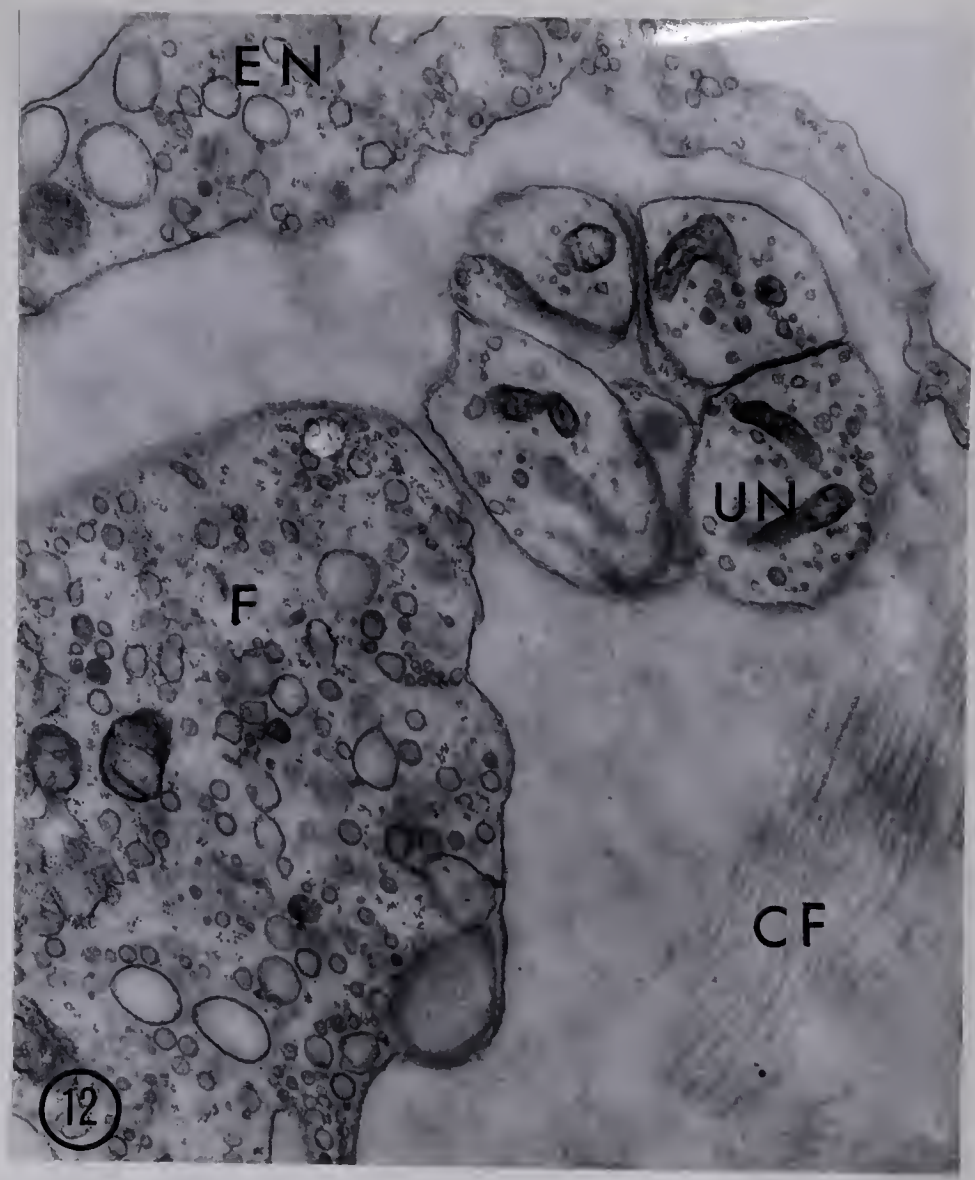
UN: Unmyelinated nerve

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 18000.

Fig. 13. Electron micrograph of a region near that shown in fig. 12.

MC: Muscular cell or "free" septa of myocardium

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 10.000.


EN
UN
F
CF
12

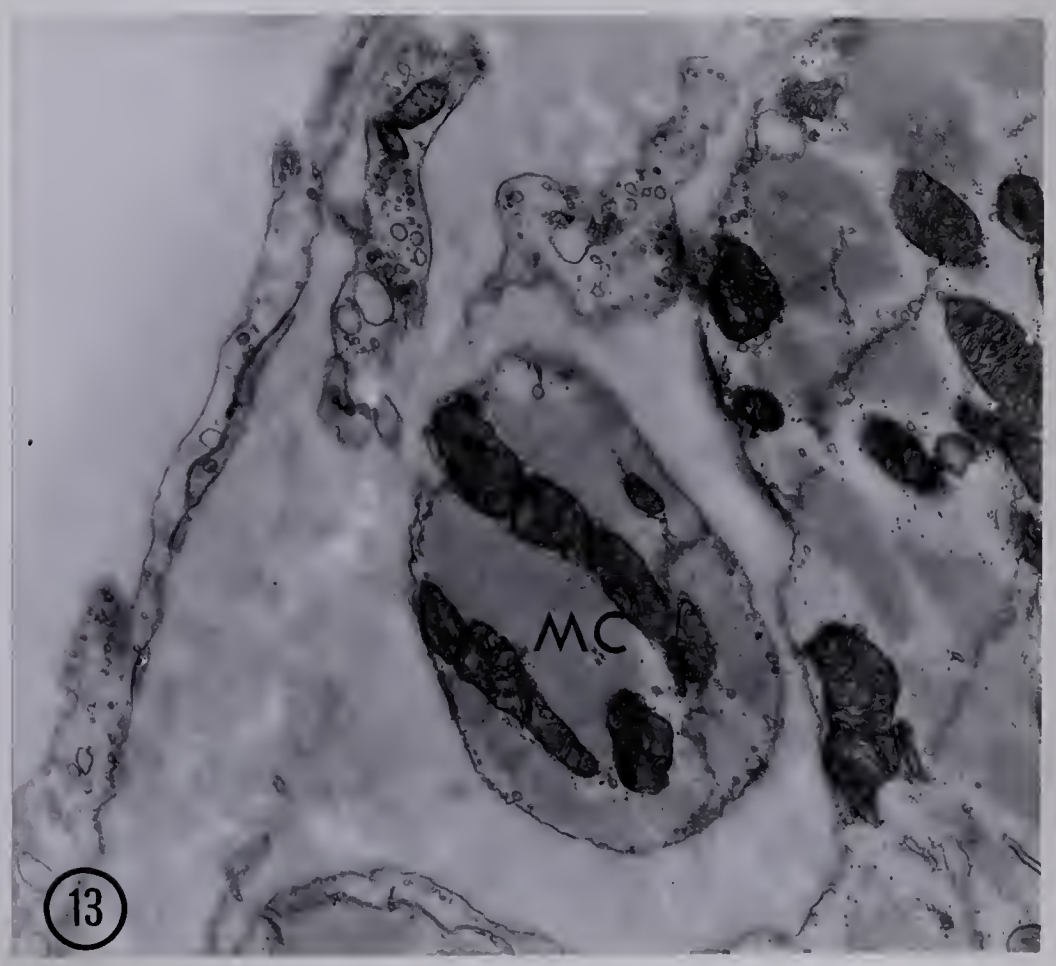
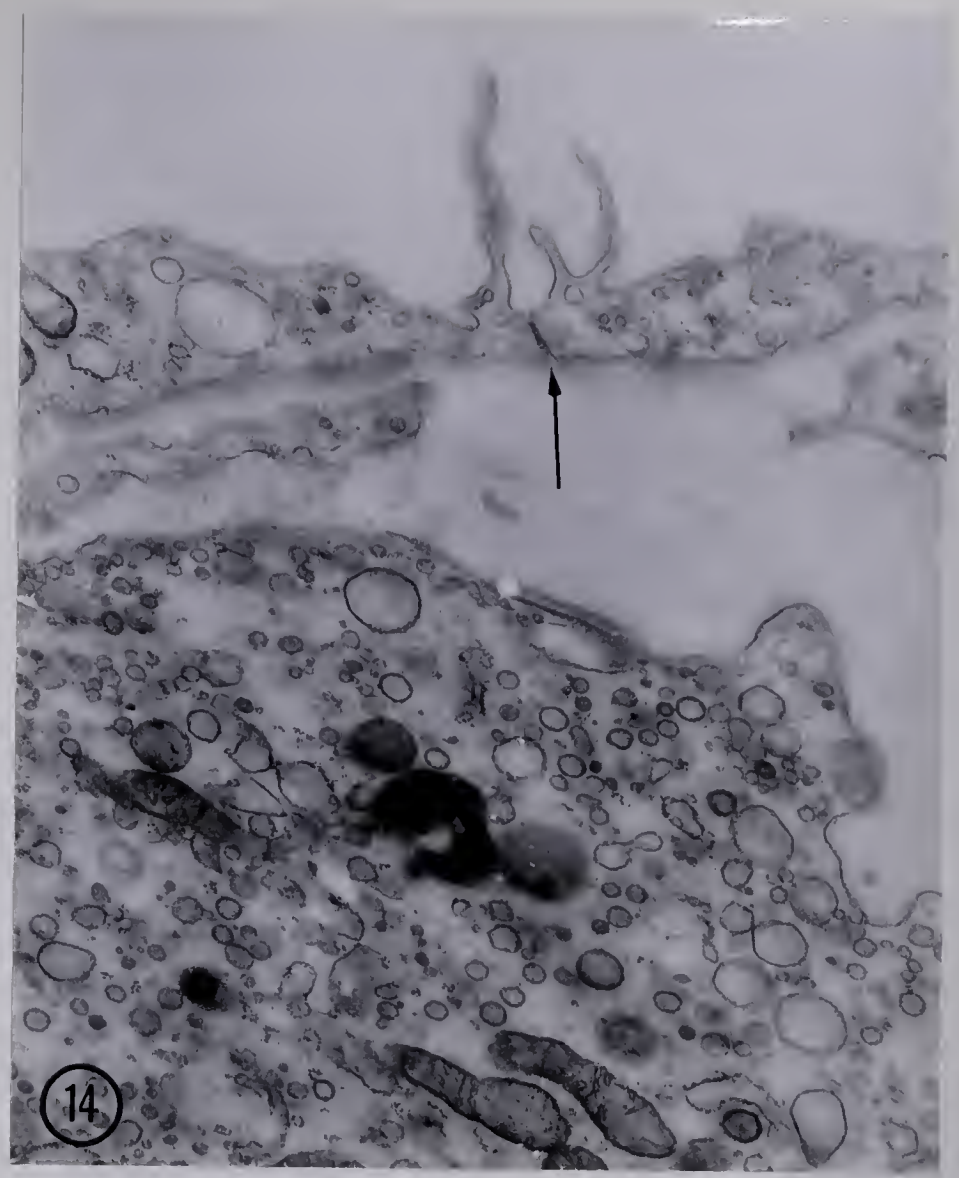

MC
13

Fig. 14. Electron micrograph demonstrating an "open" marginal fold junction in the anterior superior wall of the left atrium (arrow).
Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 18.000.

Fig. 15. Electron micrograph from a region near that shown in fig. 14, demonstrating a "closed" marginal fold junction (arrows).
Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 26.000.


14

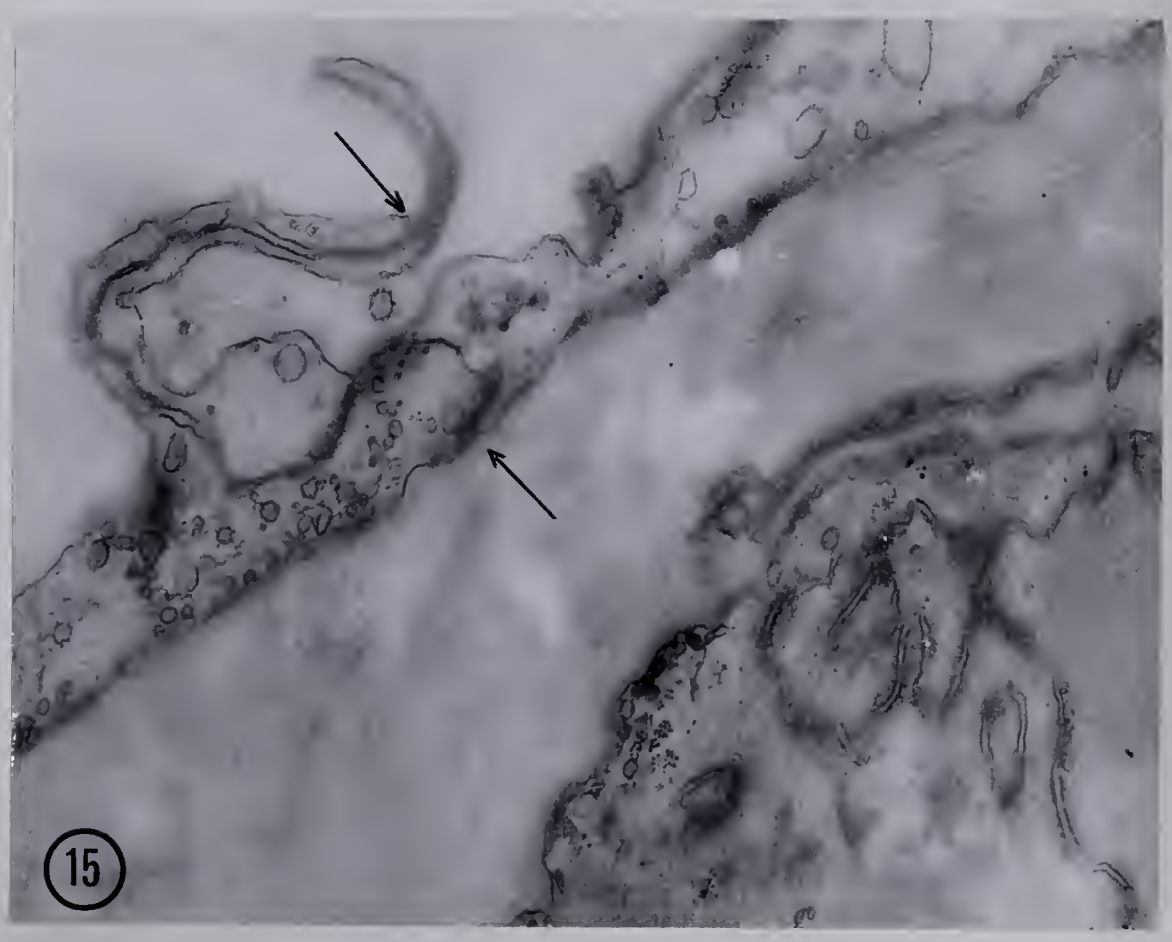
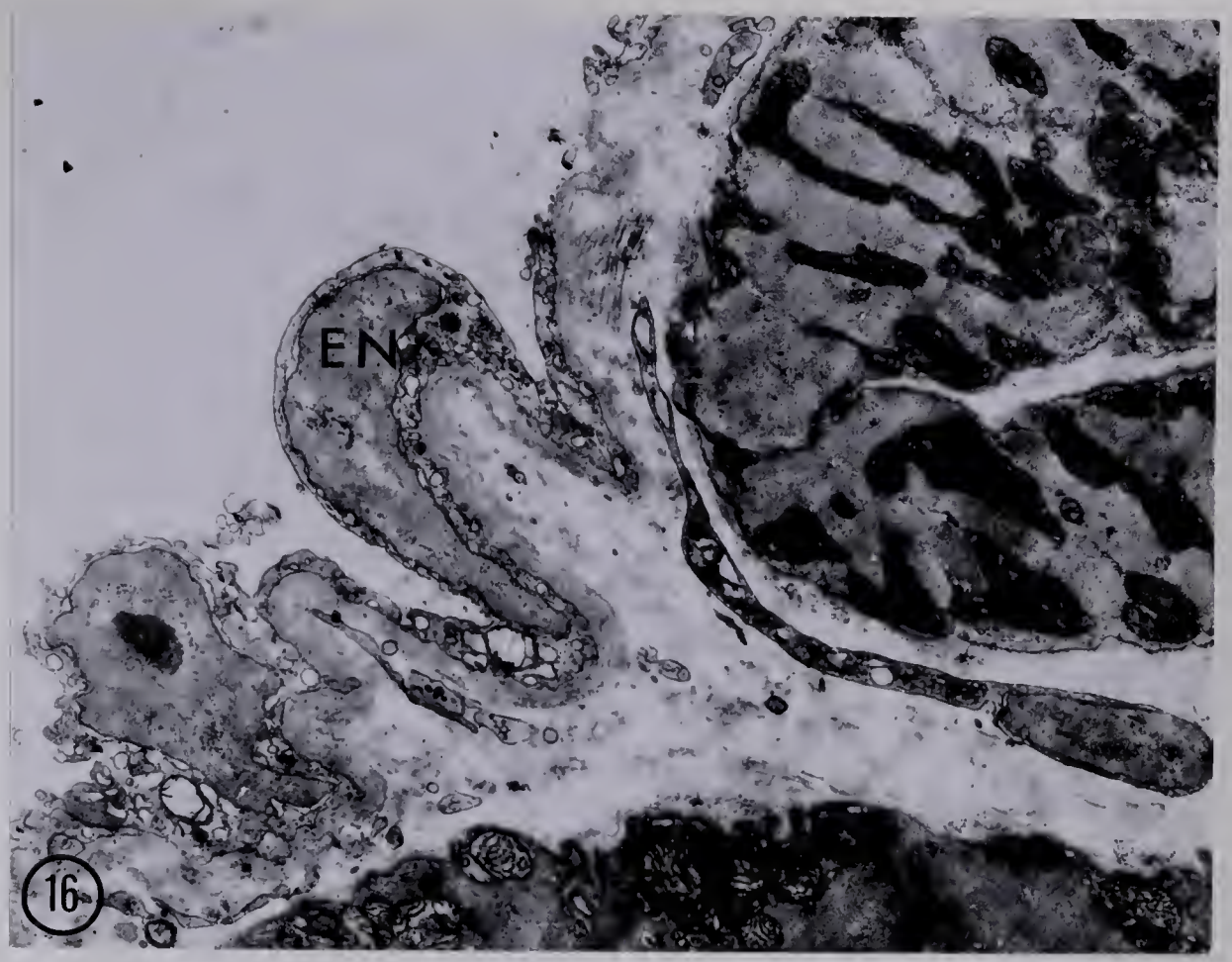

15

Fig. 16. Electron micrograph demonstrating two nuclei in the endothelium (EN) from the interventricular septum of the left ventricle.
Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 6000.

Fig. 17. Electron micrograph from the same region displayed in fig. 16. The junction between the two endothelial cells appear "V-shaped" (arrow). The basement membrane is replaced by a thicker basal lamina which measures up to several thousands Angstroms in thickness. X 18.500.


ENK
16

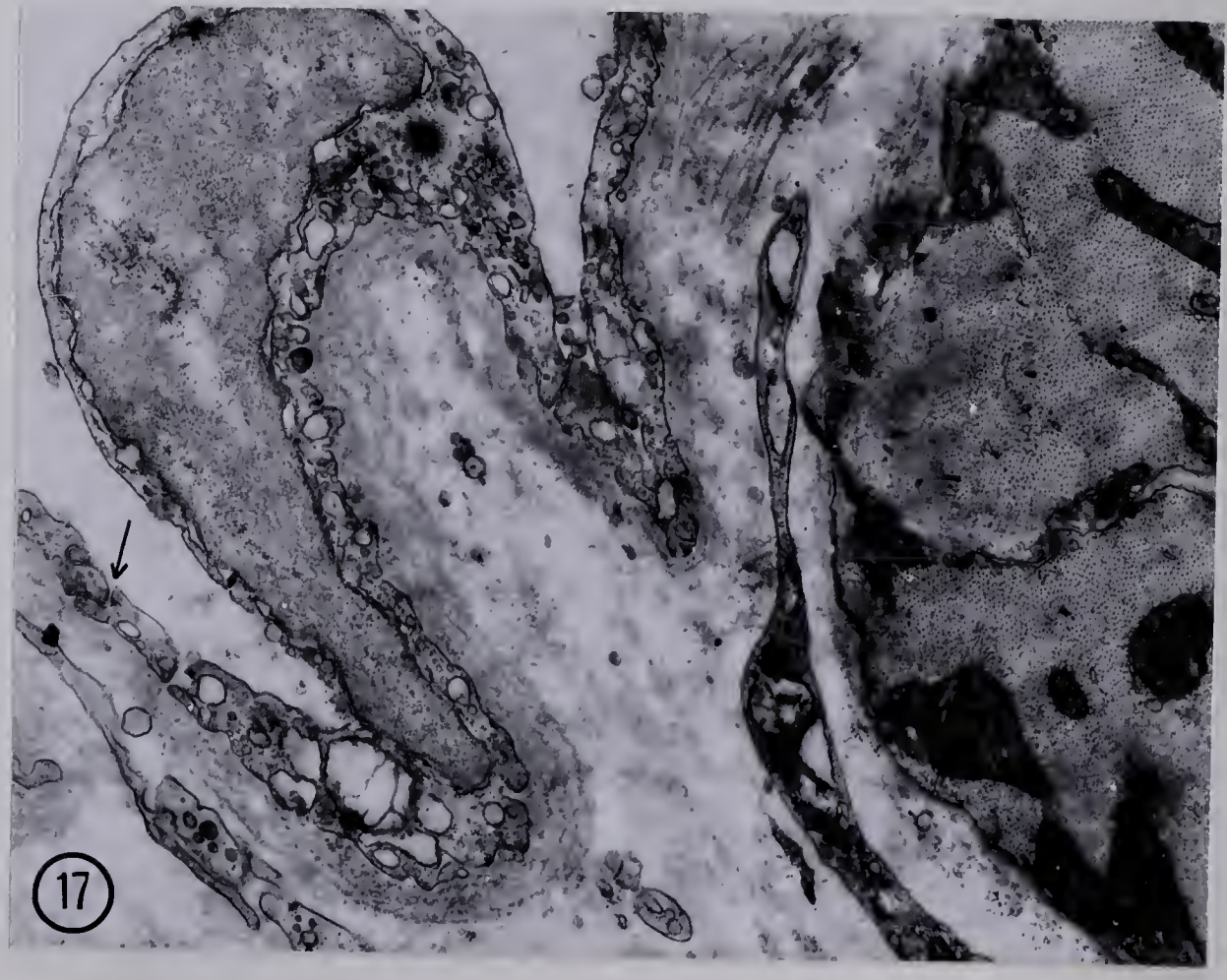

17

Fig. 18. Electron micrograph from a section approximately 1500 - 1800 Å "deeper" in the tissue than the micrographs shown in figures 16 and 17. The endothelial cell junction displayed in fig. 17 as "V-shaped" now appears to be "S-shaped", (arrows). The dark round body well shown in fig. 17 at the "upper pole" of the nucleus, which presumably is a centriole, is not displayed in fig. 18.

NU: Nucleolus

X 19500.

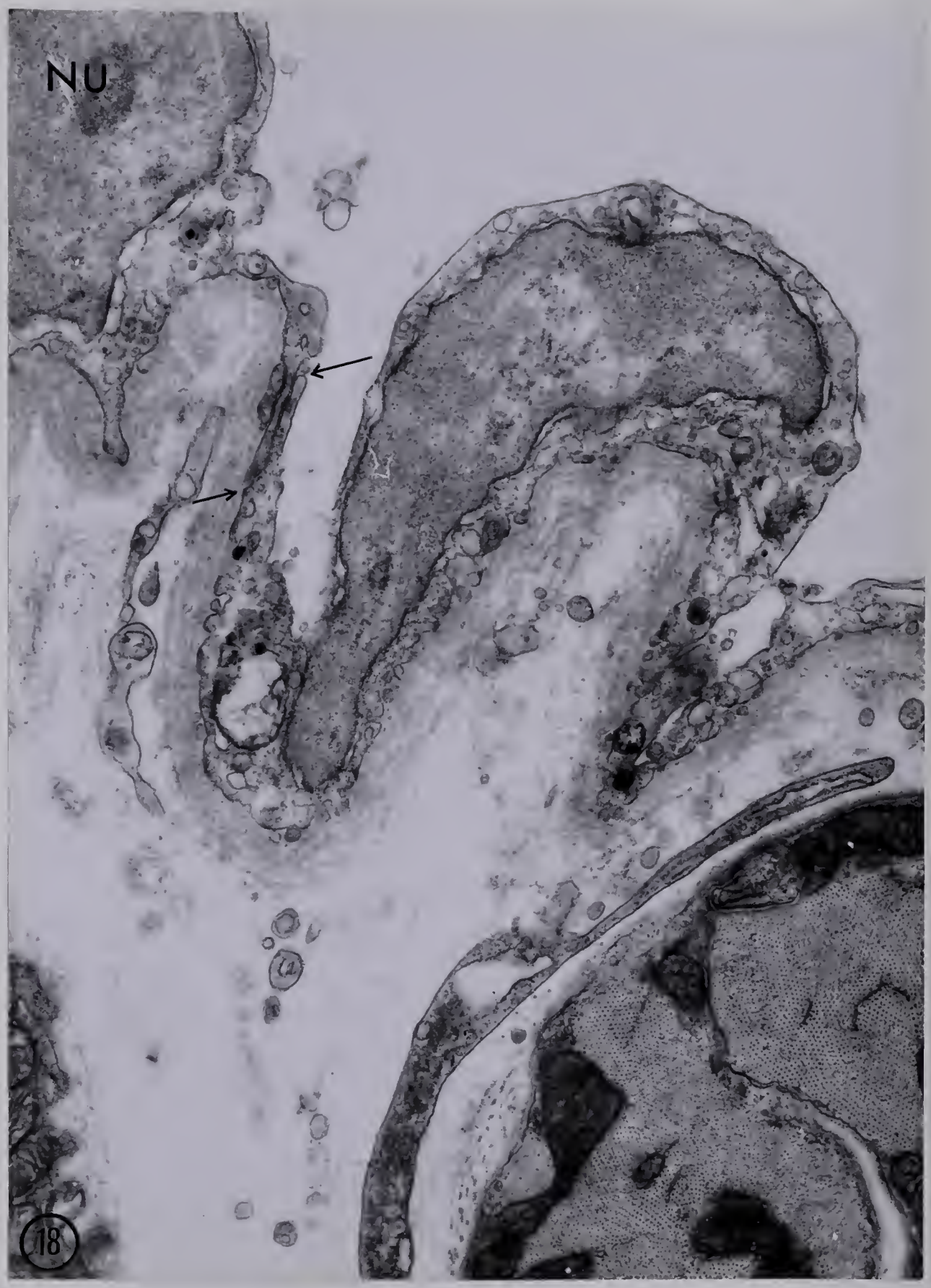

NU
18

Fig. 19. Electron micrograph showing a cross section of an unmyelinated nerve fiber in the subendocardium of the intraventricular septum of the right ventricle.

EN: Endothelium

UN: Unmyelinated nerve

Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 10.000.

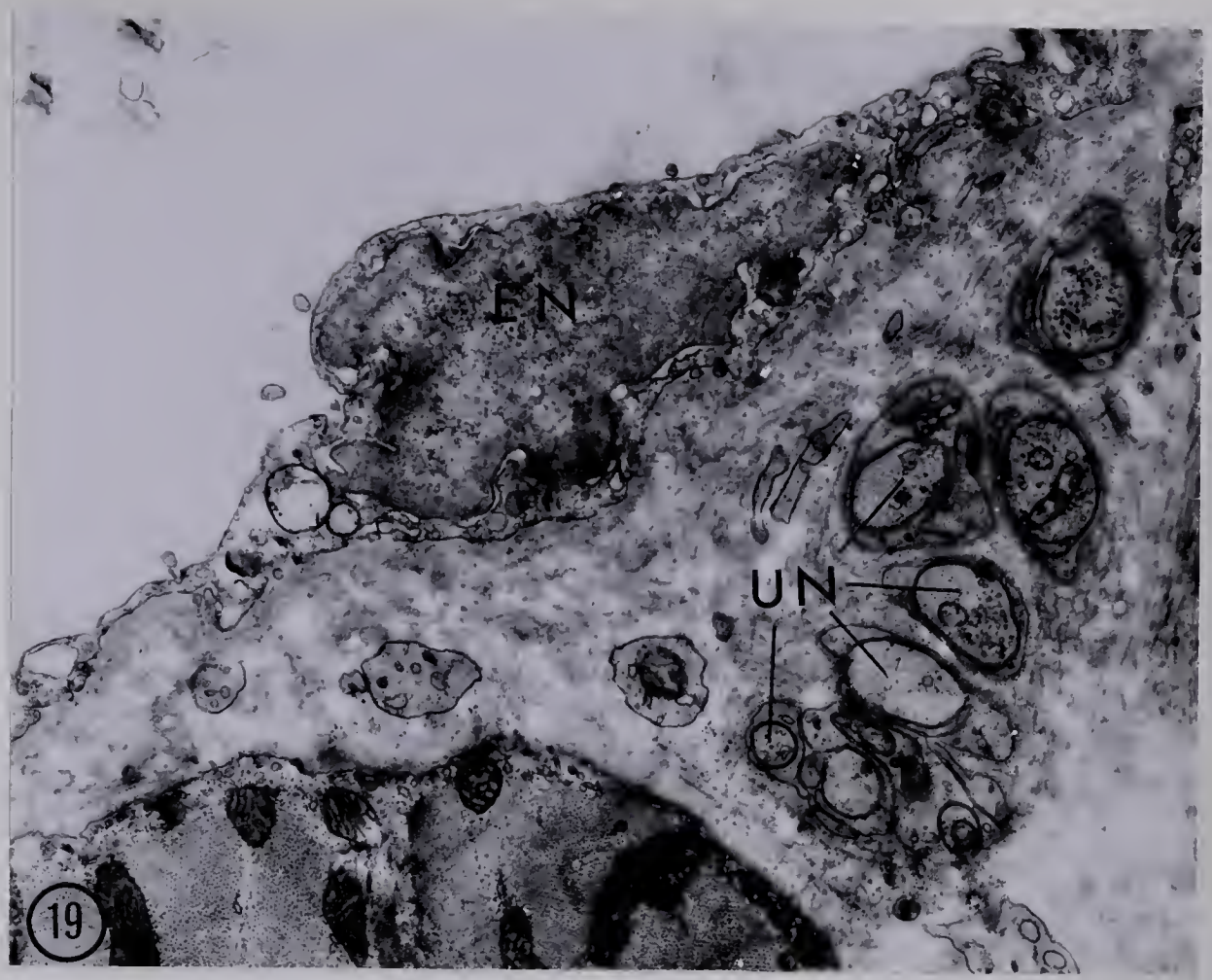
Fig. 20. Electron micrograph from the same region as fig. 19.

CF: Collagen fibers

MA: Mesaxon

SV: Synaptic vesicles

X 20.000.


EN
UN
19

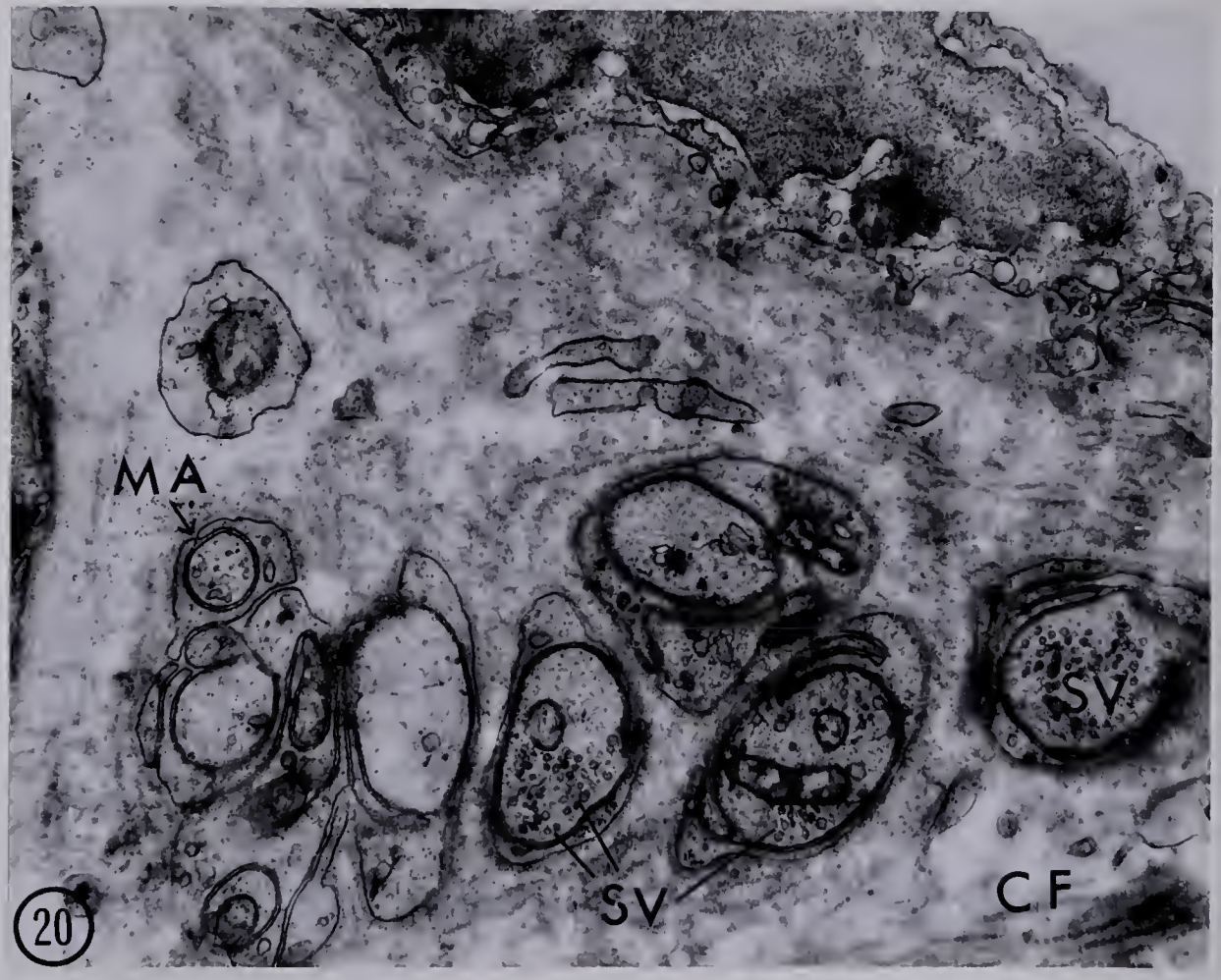

MA
SV
SV
CF
20

Fig. 21. Electron micrograph showing a capillary (C) deep within the subendothelial layer of the anterior wall of the right ventricle. More to the surface in the subendothelium is an unmyelinated nerve (UN) and portions of fibroblasts processes.

E: Red blood cell

LY: Lysosome

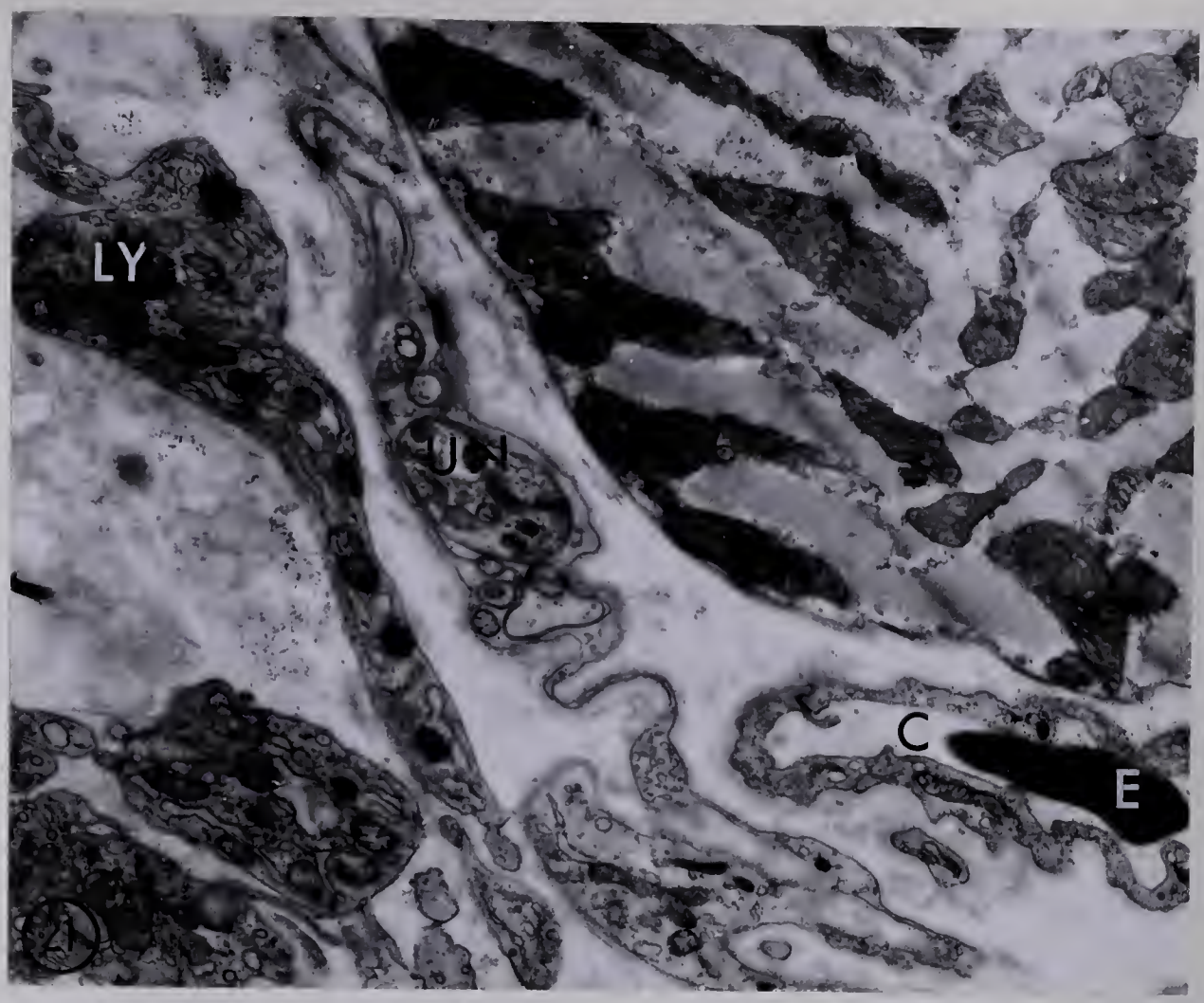
Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 10.000.

Fig. 22. Electron micrograph from the same region as that displayed in figure 21. The measaxon of the unmyelinated nerve fiber is demonstrated.

BM: "Basement membrane" coat

SV: Synaptic vesicles

X 20.000.


LY
JN
C
E
21

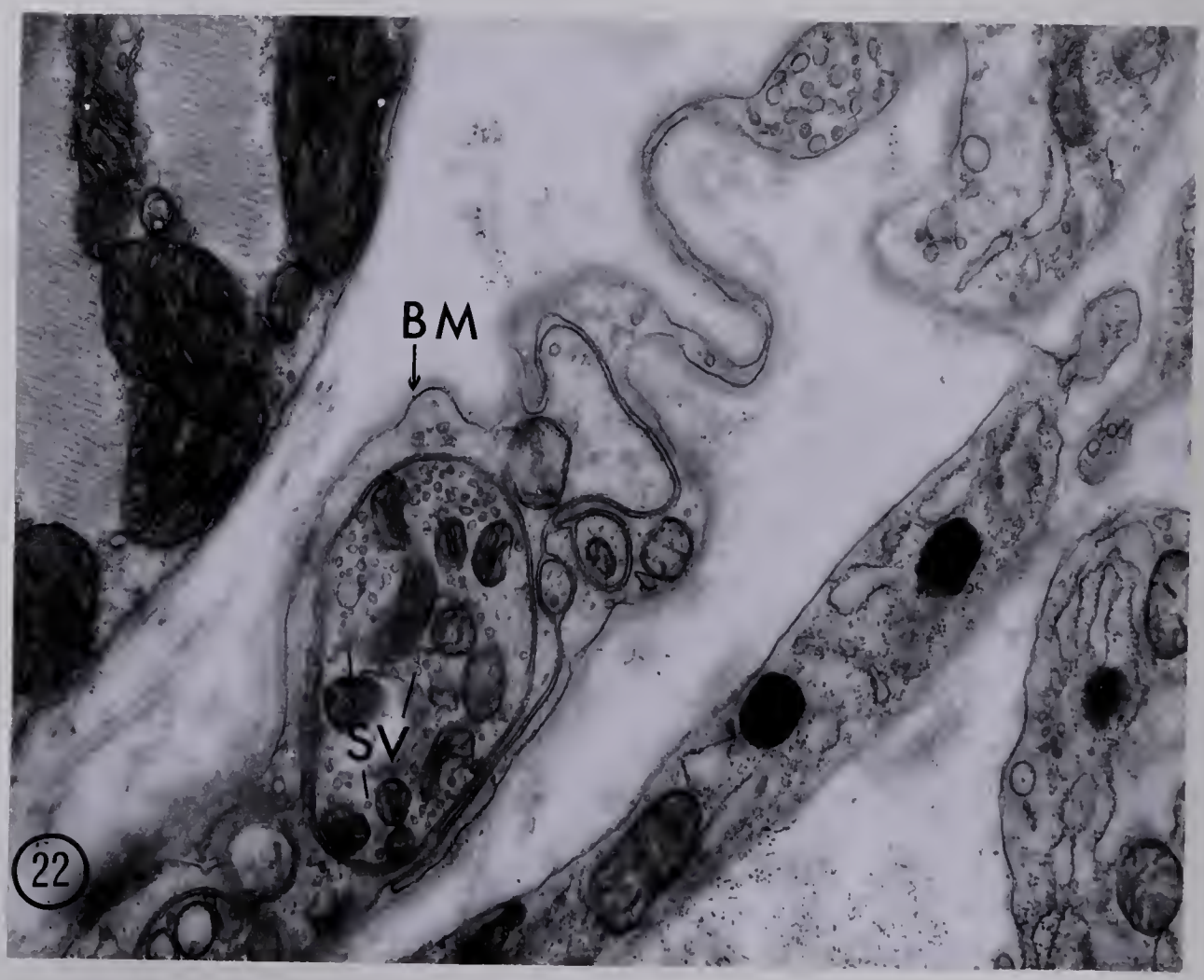

BM
SV
22

Fig. 23. Electron micrograph from a section of endocardium in the wall of the apical region of the left ventricle.

BM: Basement membrane

EN: Endothelium

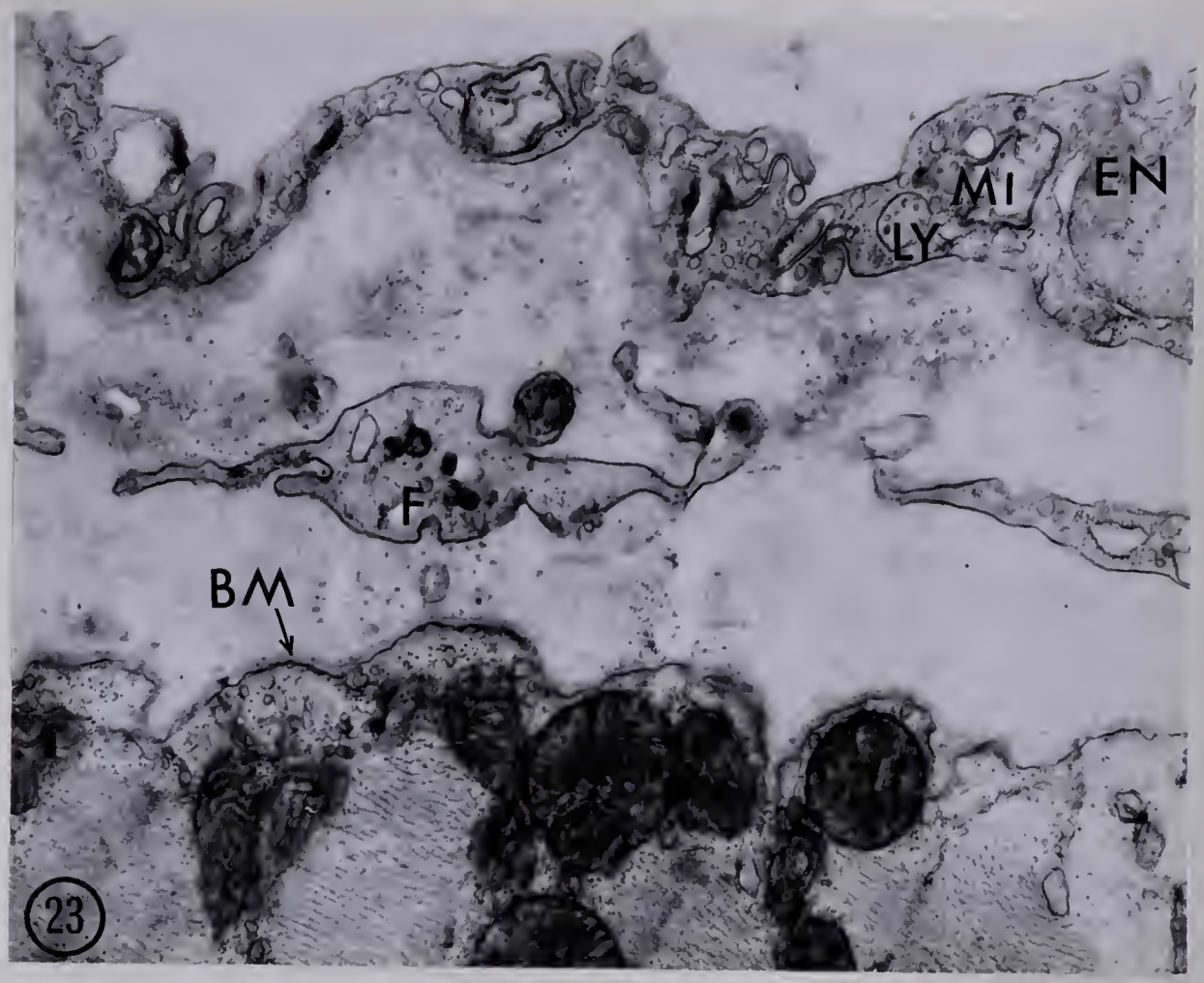
LY: Lysosome

MI: Mitochondrion

Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 24000.

Fig. 24. Electron micrograph from a section close to that displayed in fig. 23. It demonstrates a Wedge-shaped junction between two adjacent endothelial cells. Deep invagination of the sarcolemma appears at regular intervals opposite the Z-lines of the myocardium.

Osmium tetroxide fixation, maraglas embedding and lead citrate stain. X 23000.


MI
EN
LY
F
BM
23

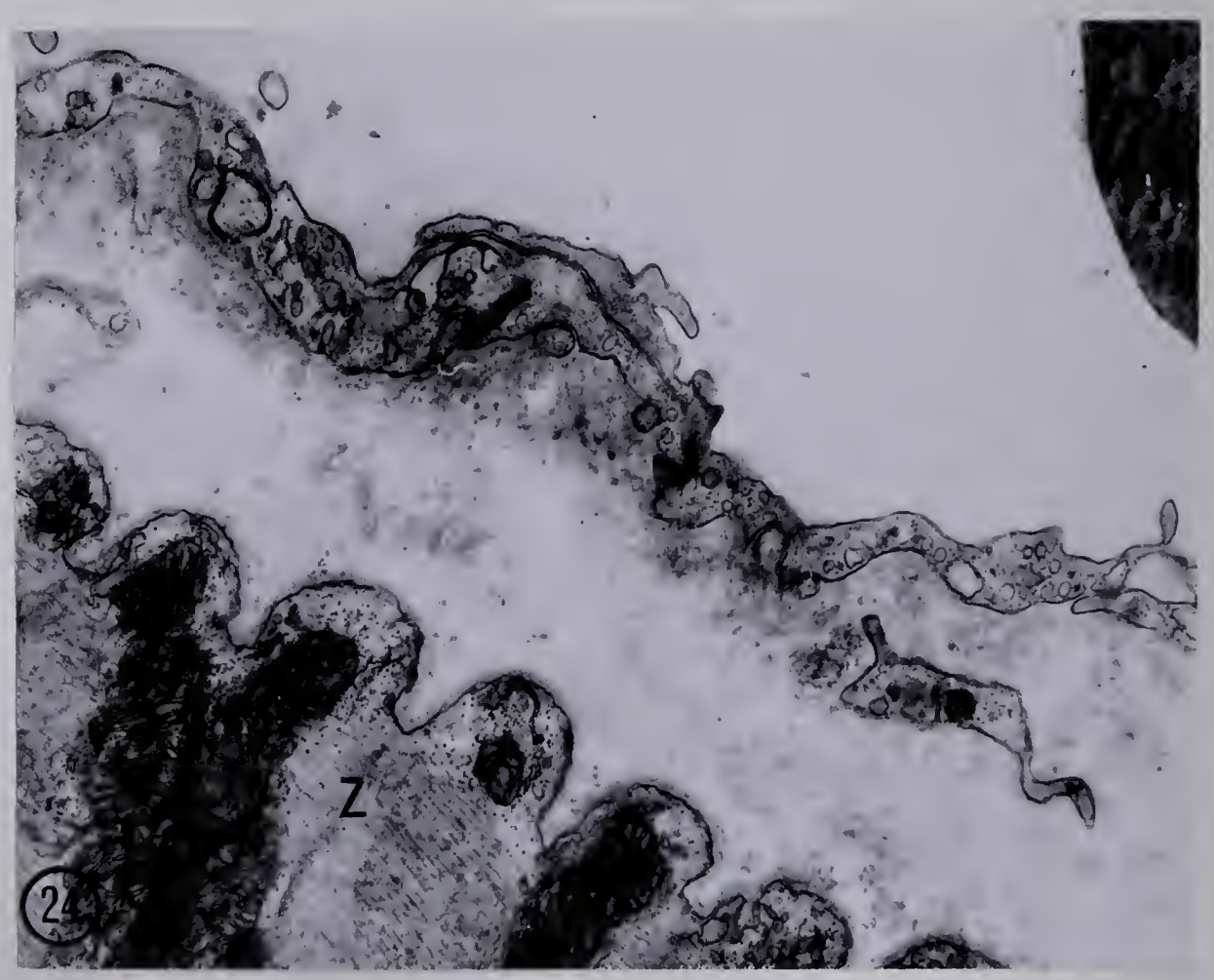
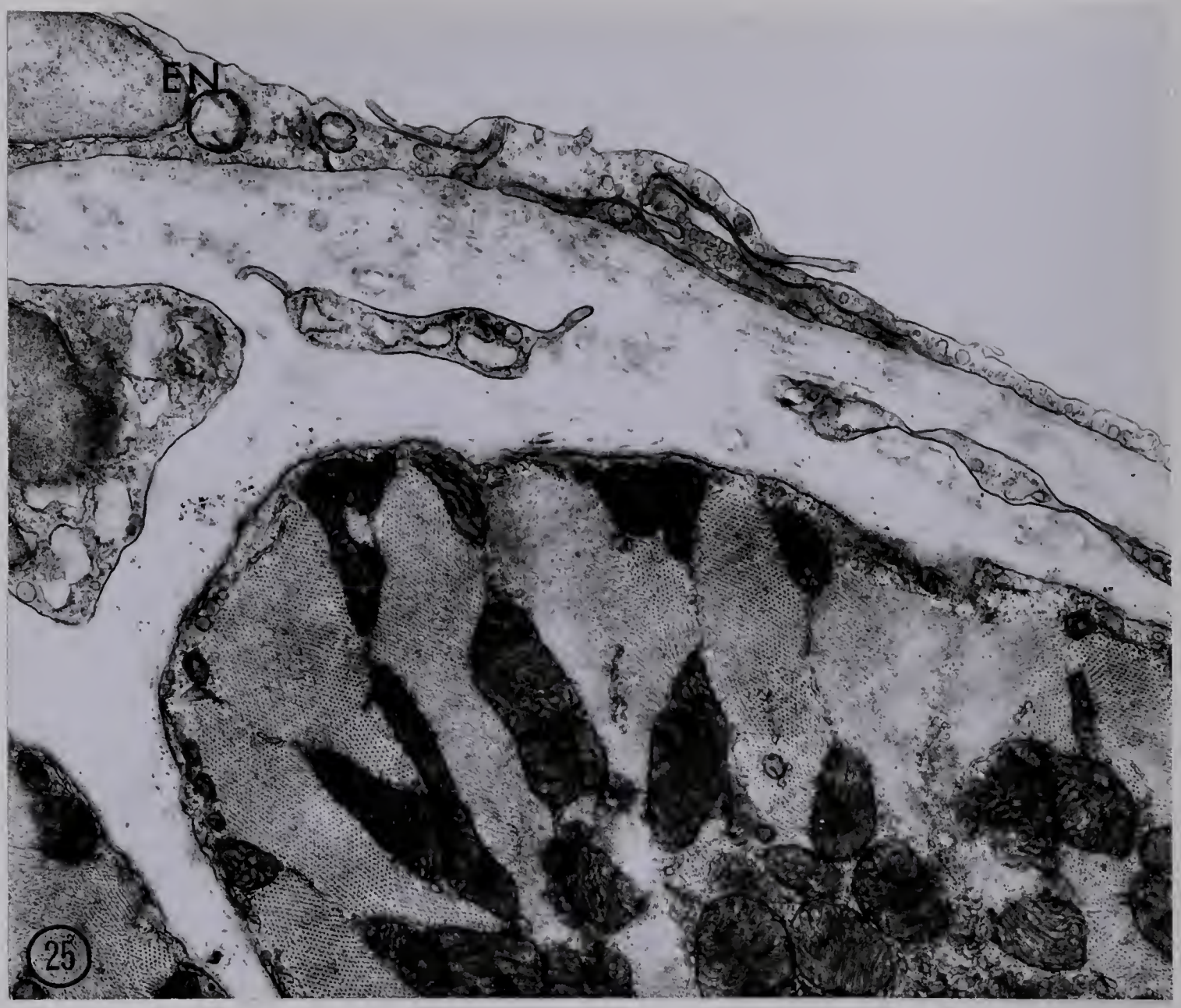

Z
24

Fig. 25. Electron micrograph demonstrating overlapping, membranous folds and interdigitation between two endothelial cells in the anterior wall of the right ventricle.
Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 13.000.

Fig. 26. Electron micrograph showing the same cell boundaries as displayed in fig. 25.
X 42.000.


EN
25


26

Fig. 27. Electron micrograph showing an indented endothelial nucleus in the apical region of the right ventricle.

EN: Endothelium

MI: Mitochondria in myocardium

Glutaraldehyde - Osmium fixation, maraglas embedding, lead citrate and uranyl acetate staining. X 19,000.

Fig. 28. Electron micrograph from a region near to that displayed in fig. 27.

F: Fibroblast

MI: Mitochondria

Glutaraldehyde Osmium fixation, maraglas embedding, lead citrate and uranyl acetate staining. X 12,500.


EN
MI
MI
27

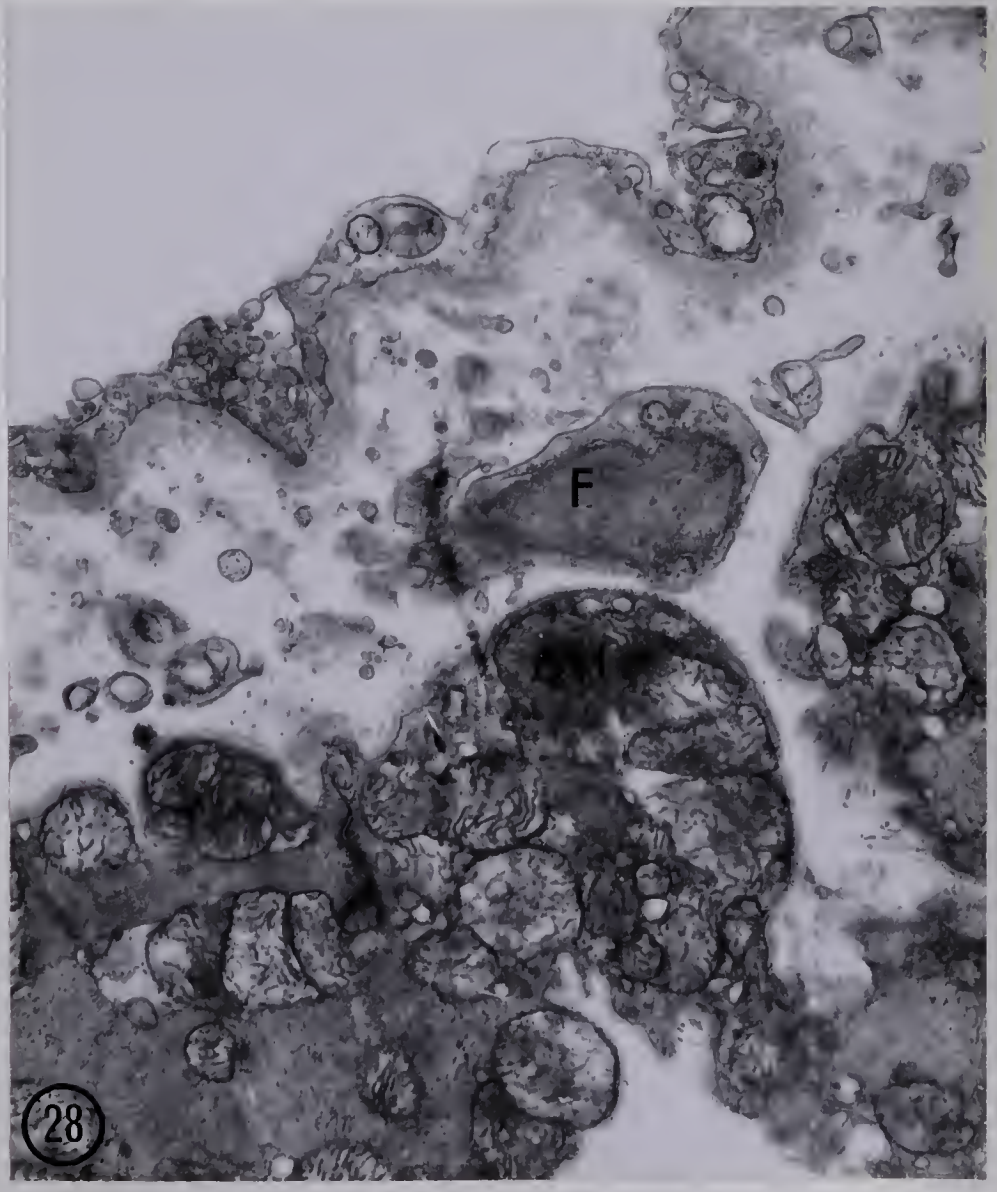

F
28

Fig. 29. Electron micrograph from a region near to that shown in figures 27 and 28. The cytoplasm of the endothelial cells is heavily packed with pinocytotic vesicles which is indicative of their function. The subendothelial layer in closest proximity to the endothelium is more electron dense than deeper within the endocardium. There it displays the basal lamina of 2000 - 3000 Å in width, which follows the infoldings of the endothelium.

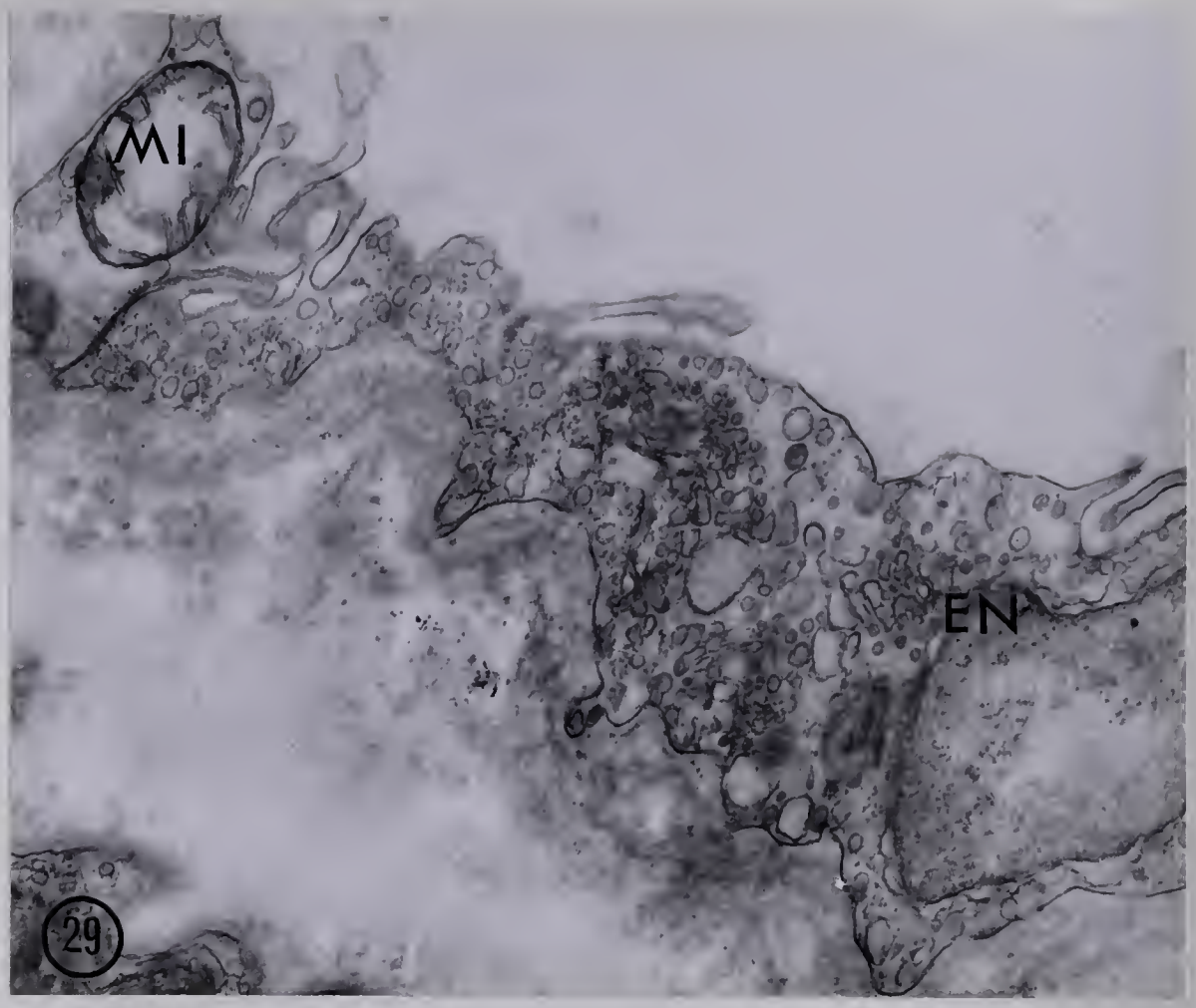
MI: Mitochondria

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate stain. X 26000.

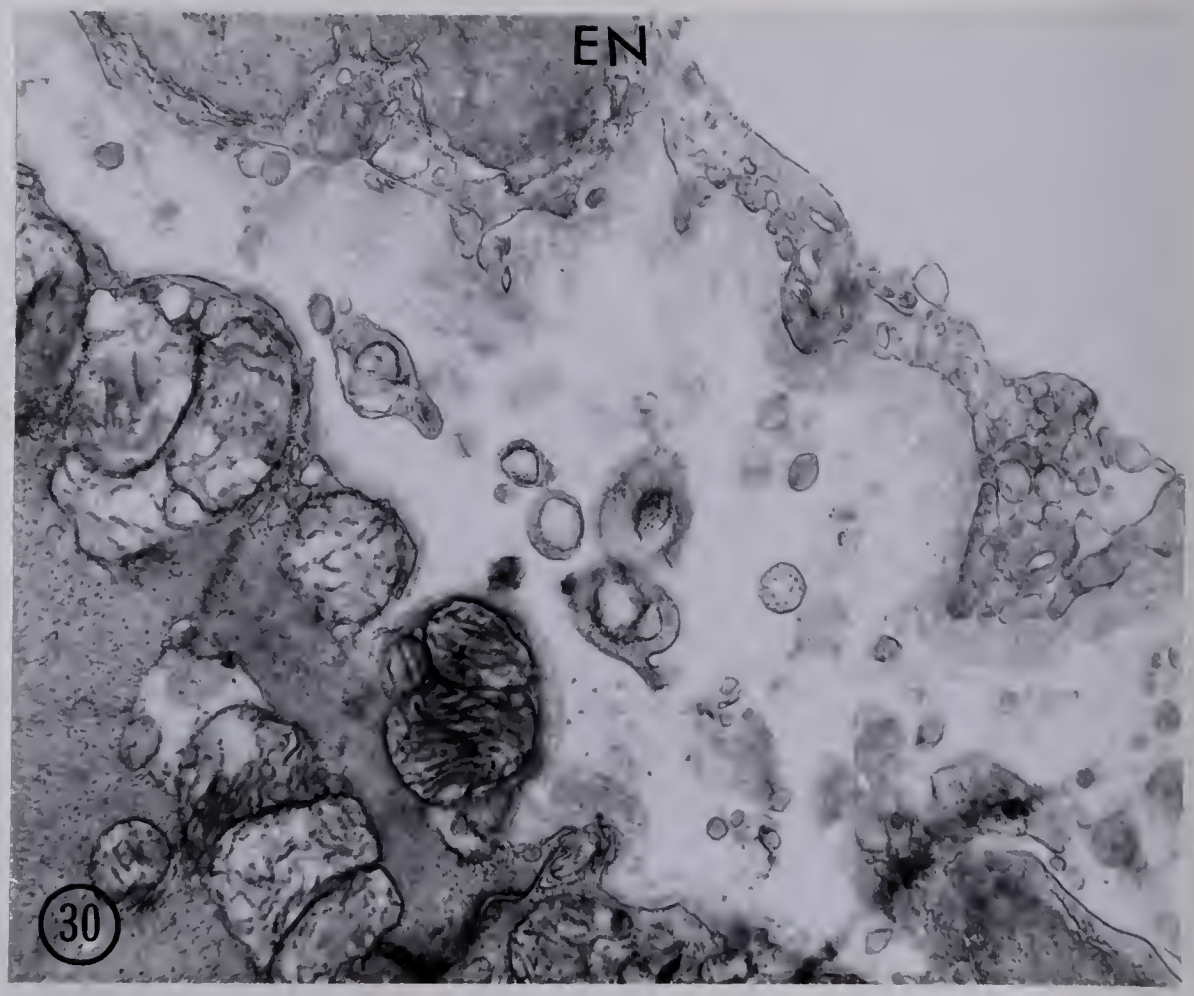
Fig. 30. Electron micrograph from a close regions illustrated in figures 27 - 29.

EN: Endothelium

Glutaraldehyde - Osmium fixation maraglas embedding and lead citrate stain. X 22.000.



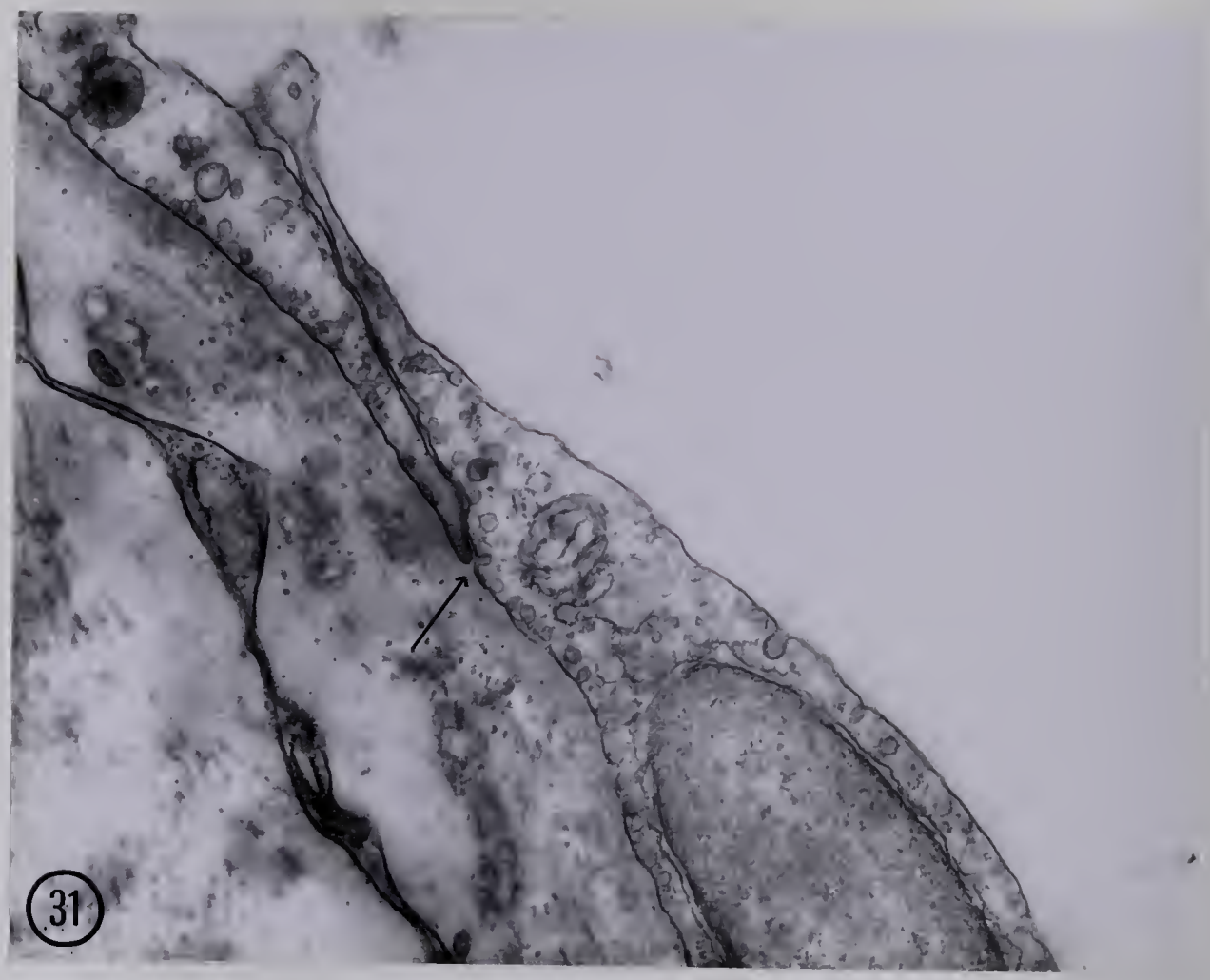
29



30

Fig. 31. Electron micrograph demonstrating a "wedge-shaped" junction (arrow) between two adjacent endothelial cells in the lateral wall of the right ventricle.
Osmium tetroxide fixation, maraglas embedding, Indium trichloride and lead citrate stain. X 24000.

Fig. 32. Electron micrograph showing a similar cell junction as displayed in fig. 31. The micrograph is from a region near to that illustrated in fig. 31.
Osmium tetroxide fixation, maraglas embedding, Indium trichloride and lead citrate stain. X 22000.


31

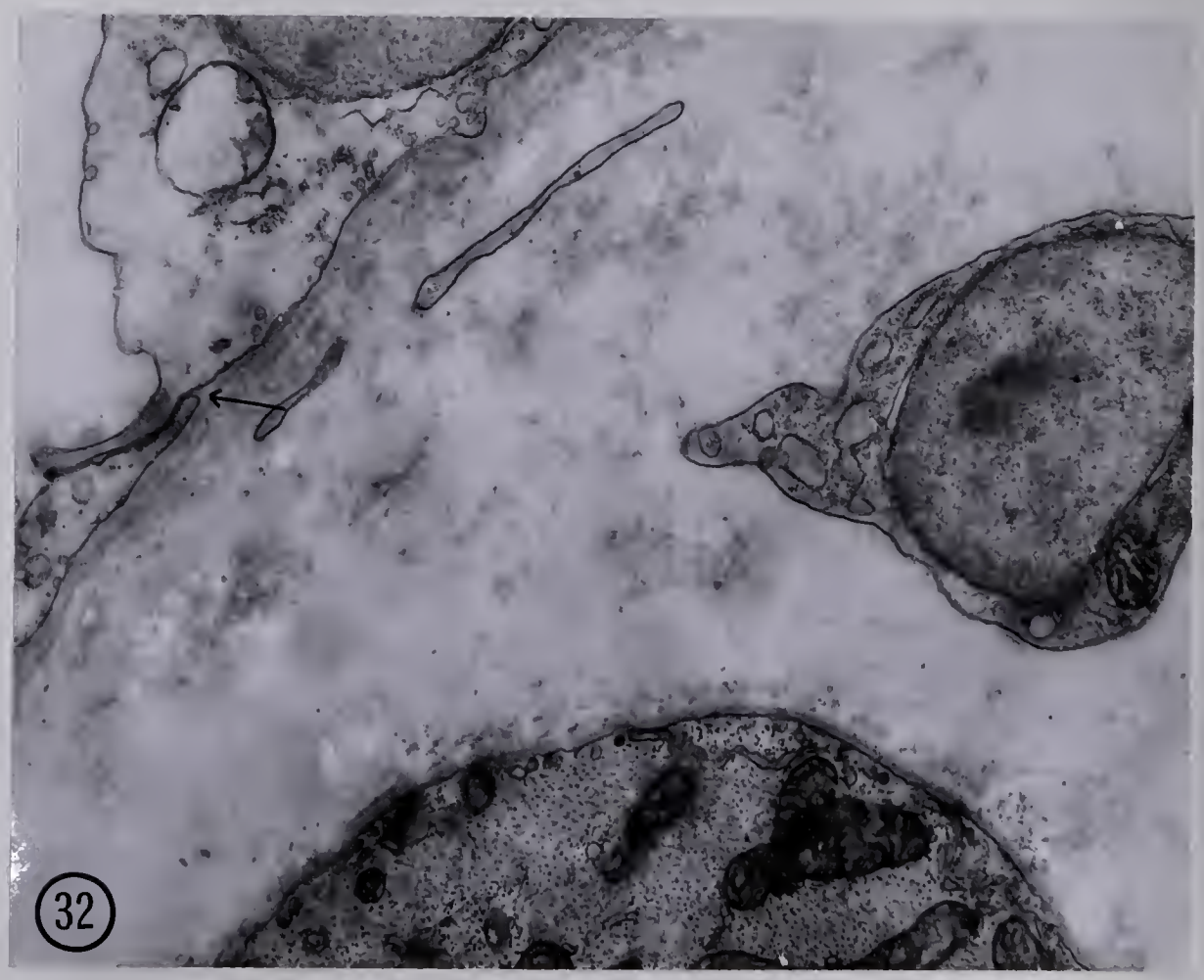

32

Fig. 33. Electron micrograph demonstrating an intercalated disc in the interventricular septum of the left ventricle. Three structural variants of the intercalated disc are illustrated. The endocardium (EN) appears to invaginate at the region towards the intercalated disc.

D: Desmosome like bodies in the intercalated disc

ZA: Intermediary junction (zonula adhaerens).

ZO: Tight junction (zonula occludens)

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 28000.

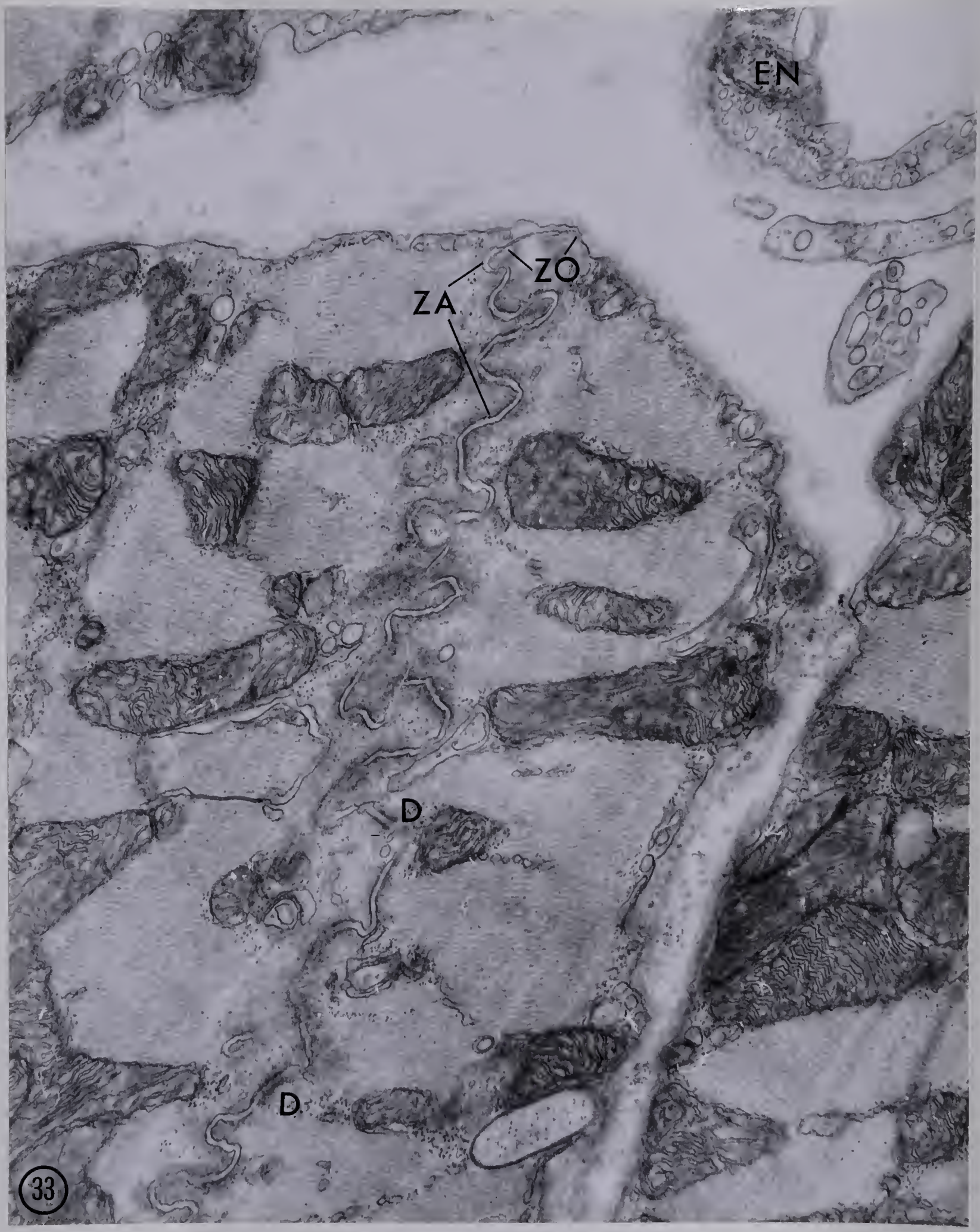
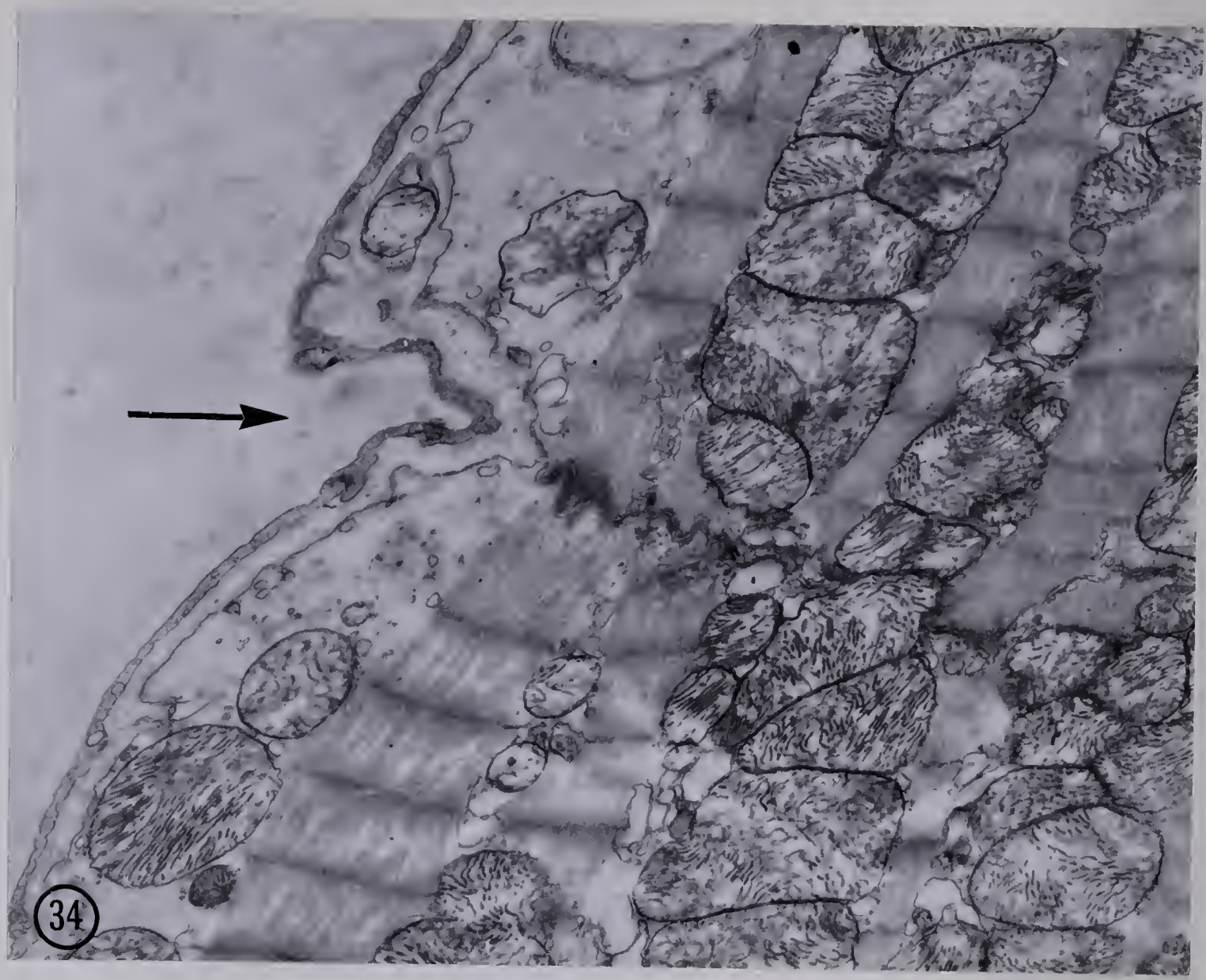

EN
ZO
ZA
D
D
33

Fig. 34. Electron micrograph demonstrating an infolding of the endocardium at the intercalated disc region in the lateral wall of the right ventricle.
The endocardium appears to be extremely thin especially in many regions in the ventricles.
Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 8500.

Fig. 35. Electron micrograph taken from the lateral wall of the right ventricle. The distance between the adjacent sarcolemmas at the endo-myocardial junction is approximately 120 - 140 Å
The gap is closed by the basement membrane as it passes over it. (arrow).
Osmium tetroxide fixation, maraglas embedding and lead citrate staining. X 16.000.


34

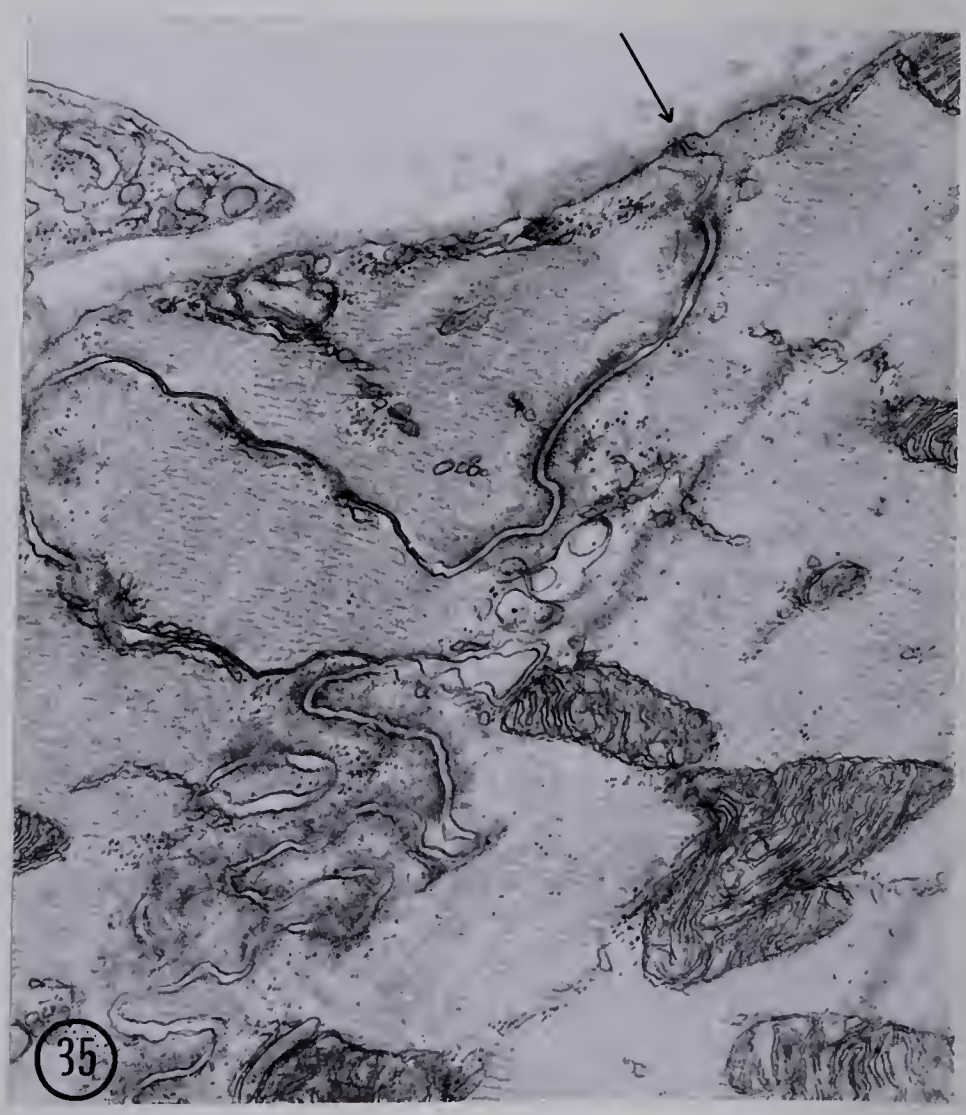

35

Fig. 36. Electron micrograph demonstrating sarcoplasmic reticulum (SR) within a muscle cell from a ventricular wall. The sarcoplasmic reticulum was found in close association to the sarcolemma (fig. 41) connecting the regularly arranged mitochondria (MI) and the nucleus. The sarcoplasmic reticulum often appears somewhat twisted or "spiral shaped" tubules.

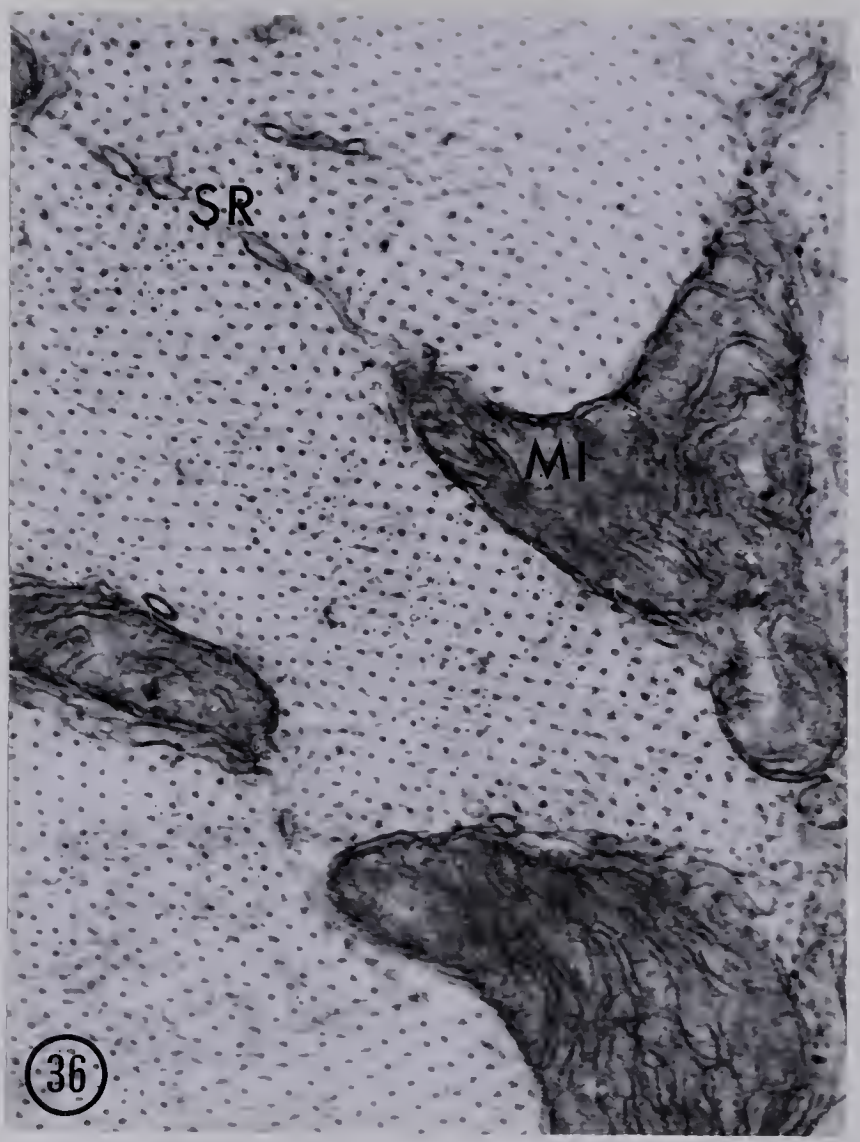
Osmium tetroxide fixation, maraglas embedding and lead citrate stain. X 50.000.

Fig. 37. Electron micrograph from a region near to that shown in fig. 36.

N: Nucleus of a myocardiac cell

SR: Sarcoplasmic reticulum

X 45000.


SR
MI
36

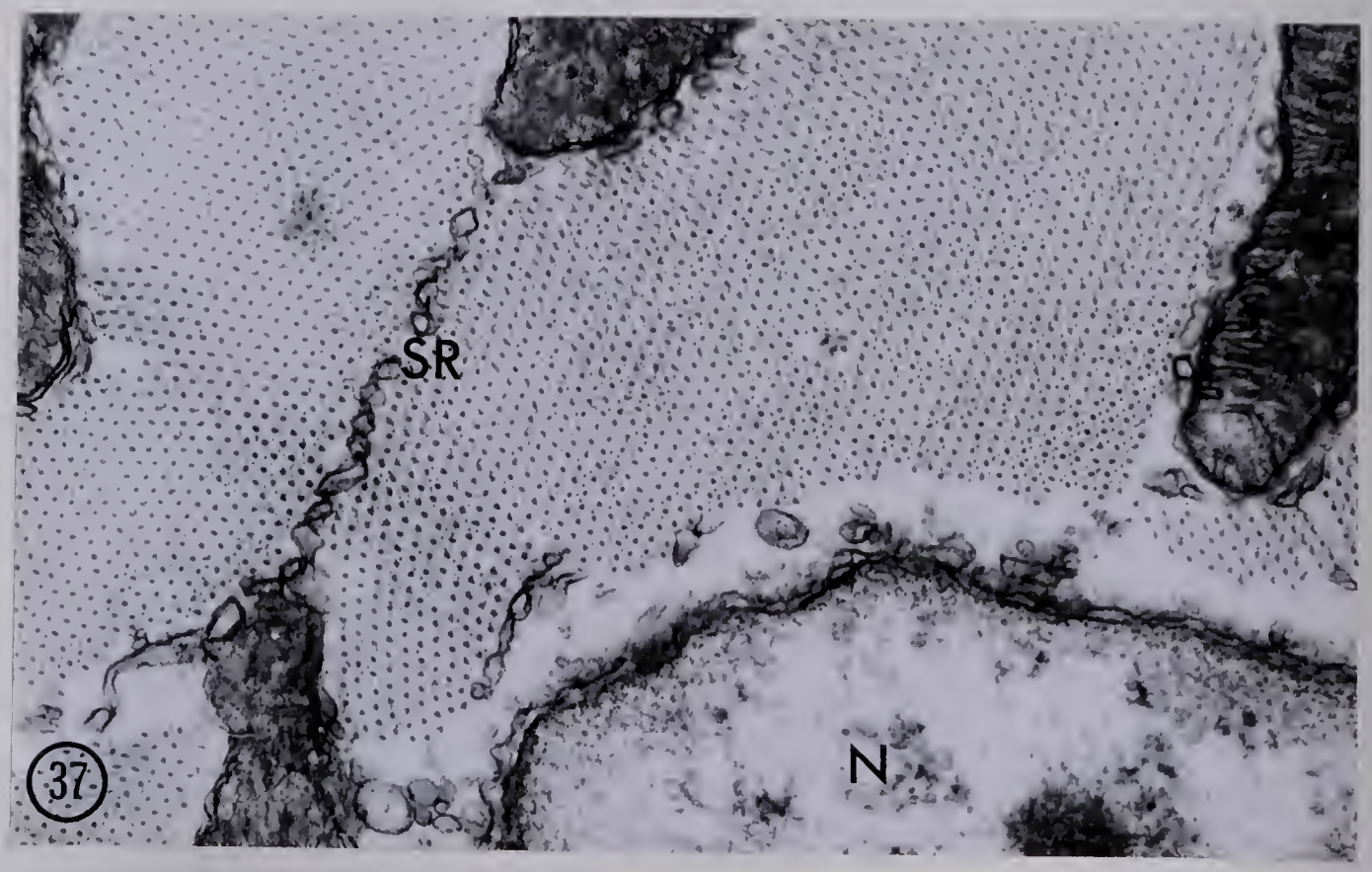

SR
N
37

Fig. 38. Electron micrograph from the lateral wall of the left ventricle demonstrating two capillaries (C) in the intercellular connective tissue space of the myocardium, and their distance from the endocardium (EN). Capillaries were only found in the deepest portion of endocardium (fig. 21).

N: Nucleus

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 7000.

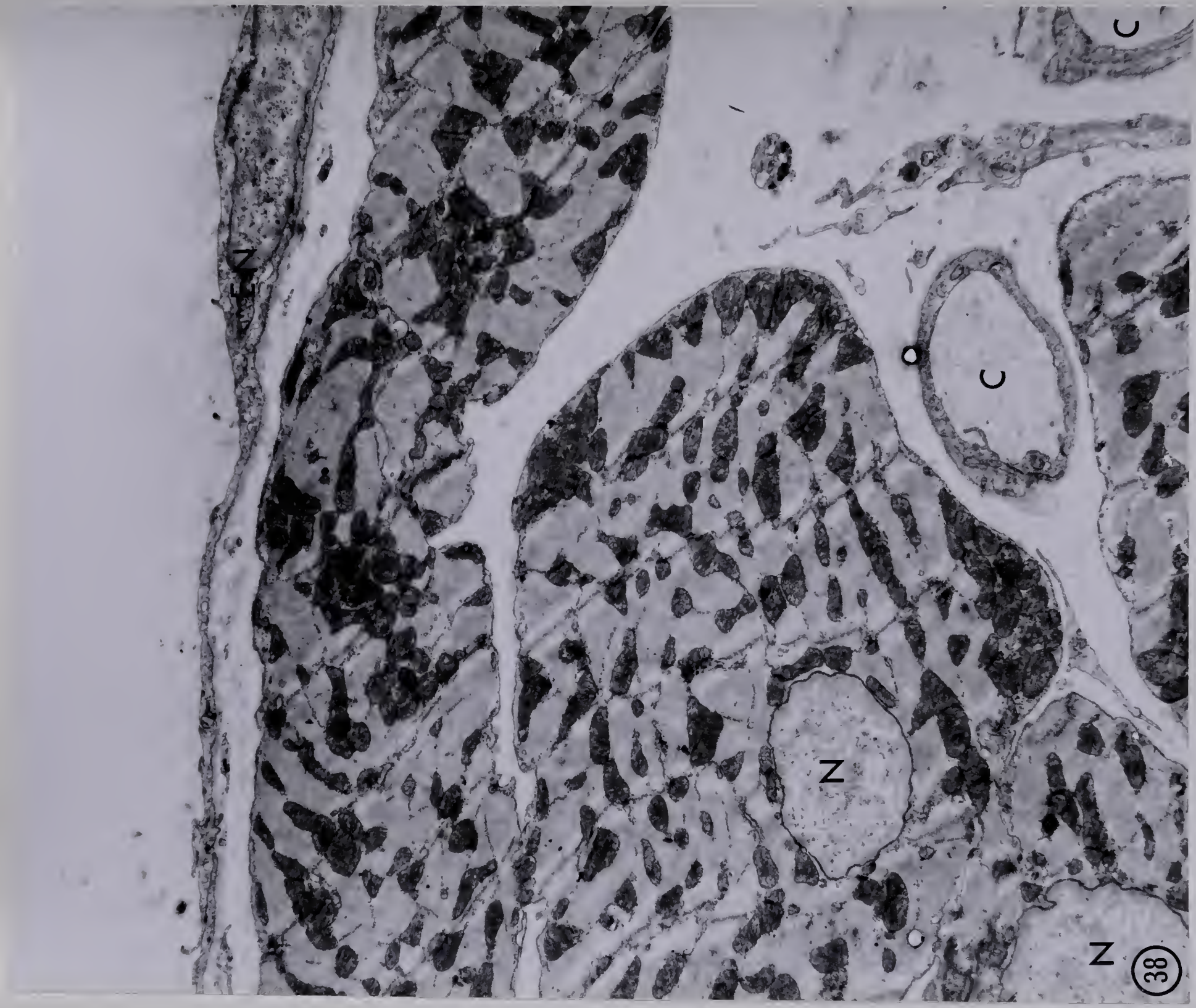

C
C
Z
Z
38

Fig. 39. Electron micrograph demonstrating an interface and an overlapping between two adjacent endothelial cells (S-shaped cell junction) in the anterior wall of the left ventricle. Numerous pinocytotic vesicles (P) are found along the base of the endothelial cells.

LY: Lysosome

SL: Sarcolemma

V: Vacuolae

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 60000.

39

Fig. 40. Electron micrograph demonstrating a longitudinal section of two centrioles (CE) in a fibroblast in the subendothelial layer of the left ventricle.

G: Golgi complex

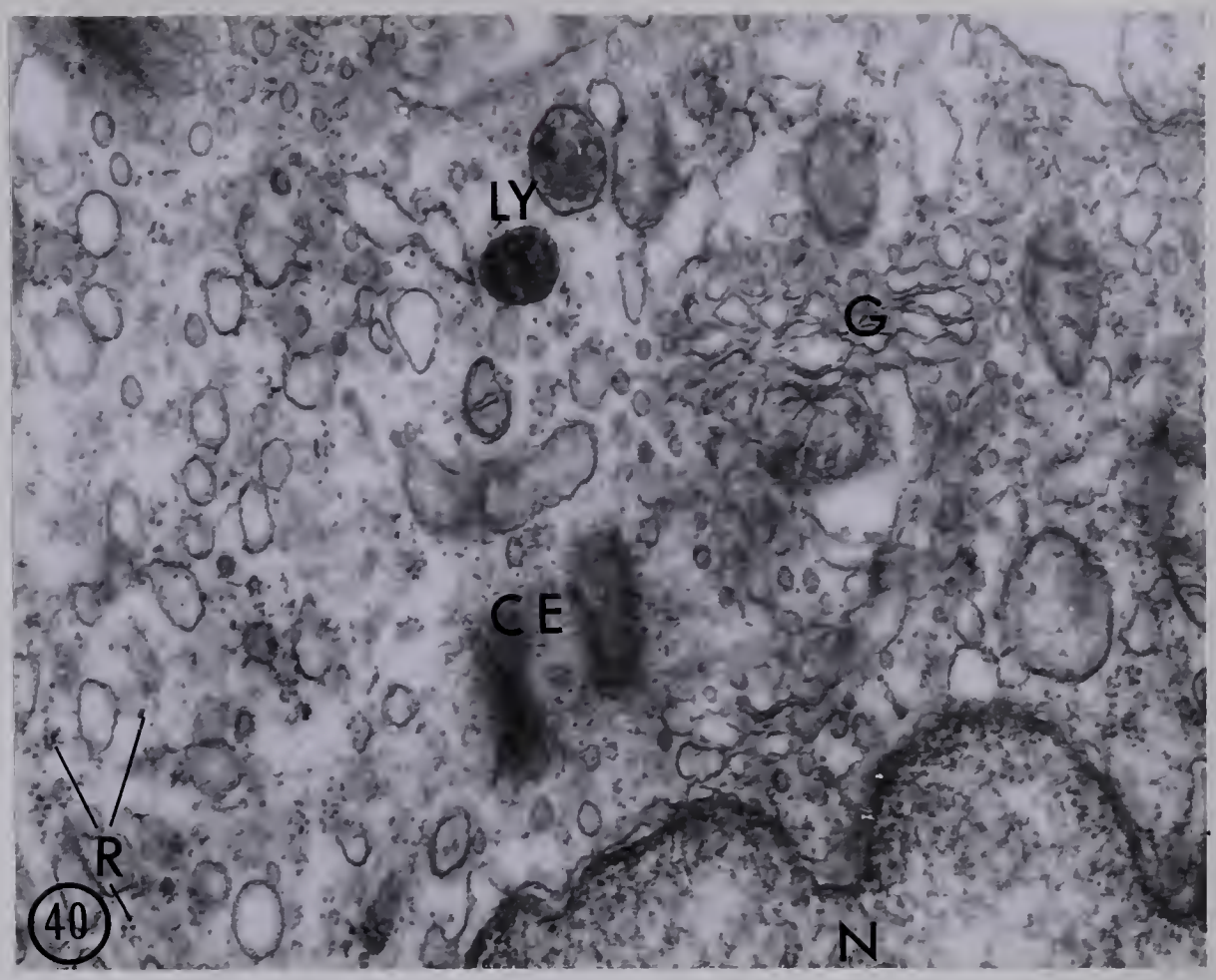
LY: Lysosomes

N: Nucleus

R: Ribosomes

Glutaraldehyde - Osmium fixation maraglas embedding and lead citrate staining. X 32000.

Fig. 41. Electron micrograph from the same region as displayed in fig. 16. Sarcoplasmic reticulum (SR) is often found in close association with the sarcolemma and mitochondria. X 58000.


LY
G
CE
R
40
N


SR
41

Fig. 42. Electron micrograph taken from the lateral wall of the left ventricle. It shows unmyelinated nerve fibers close to the endothelium (EN).

CF: Collagen fibers

MI: Mitochondria

SV: Synaptic vesicles

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 32.000.

42

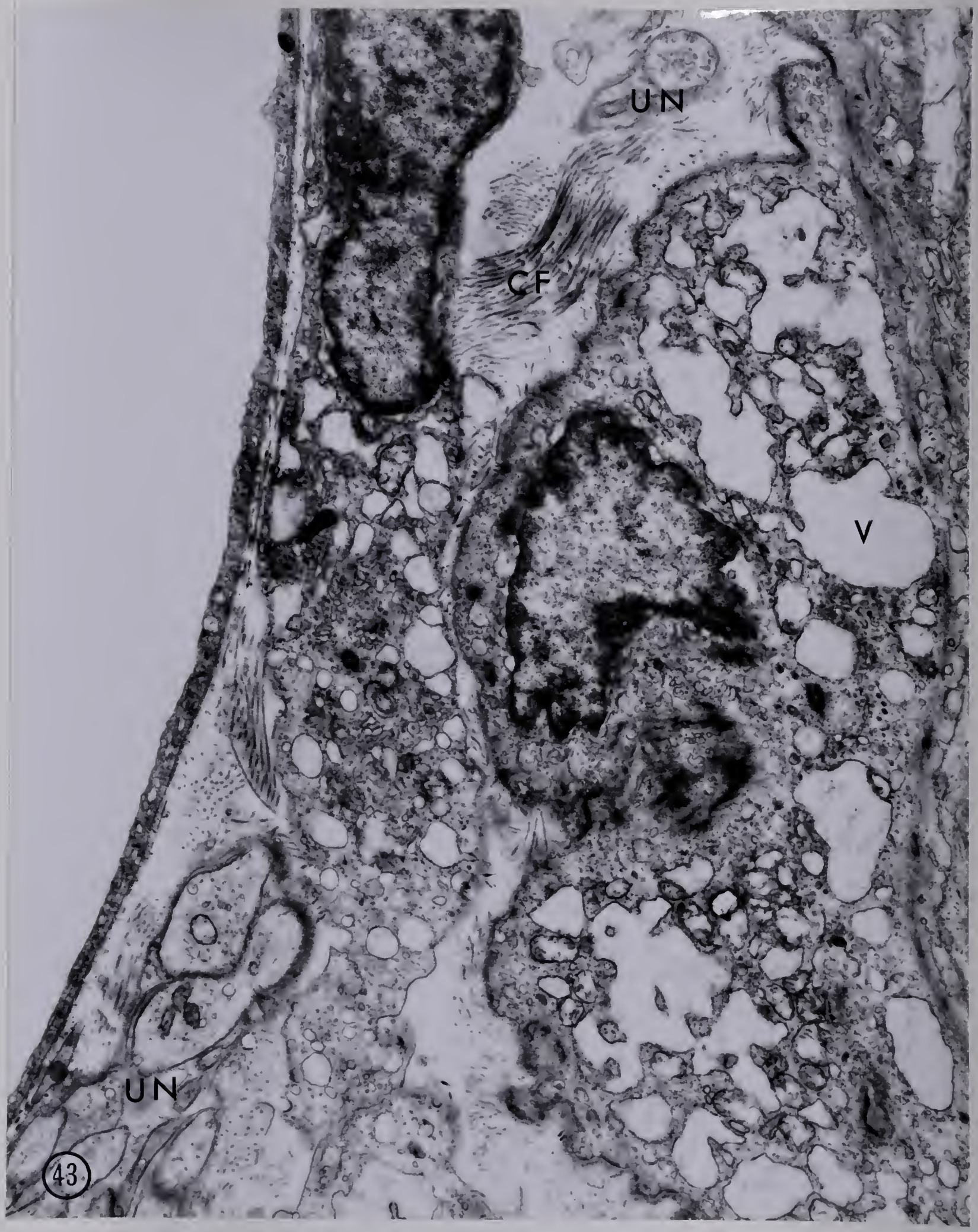
Fig. 43. Electron micrograph from the lateral wall of the left ventricle. Large irregular vacuoles are to be seen within the cytoplasm of the two fibroblasts shown.

CF: Collagen fibers

UN: Unmyelinated nerve

V: Vacuole

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 16000



43

Fig. 44. Electron micrograph of the same region as illustrated in fig. 43. The plasma membrane of the fibroblast is perforated in the region where the vacuoles are exposed to it. At the region of "open vacuoles (arrows), collagen fibrils (CF) are frequently found.

BM: Basement membrane

CE: Centriole

UN: Unmyelinated nerve fiber

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 45.000.


BM
UN
CF
V
V
CE
44

Fig. 45. Electron micrograph illustrating a centriole (CE) encircled by a Golgi complex (G). The micrograph was taken from a section of a fibroblast within the subendothelial layer of the right ventricle.

GV: Golgi vesicles

MI: Mitochondrion

N: Nucleus

R: Ribosomes

SER: Smooth endoplasmic reticulum

Glutaraldehyde - Osmium fixation, maraglas embedding, phosphotungstic acid and lead citrate stain. X 65000.

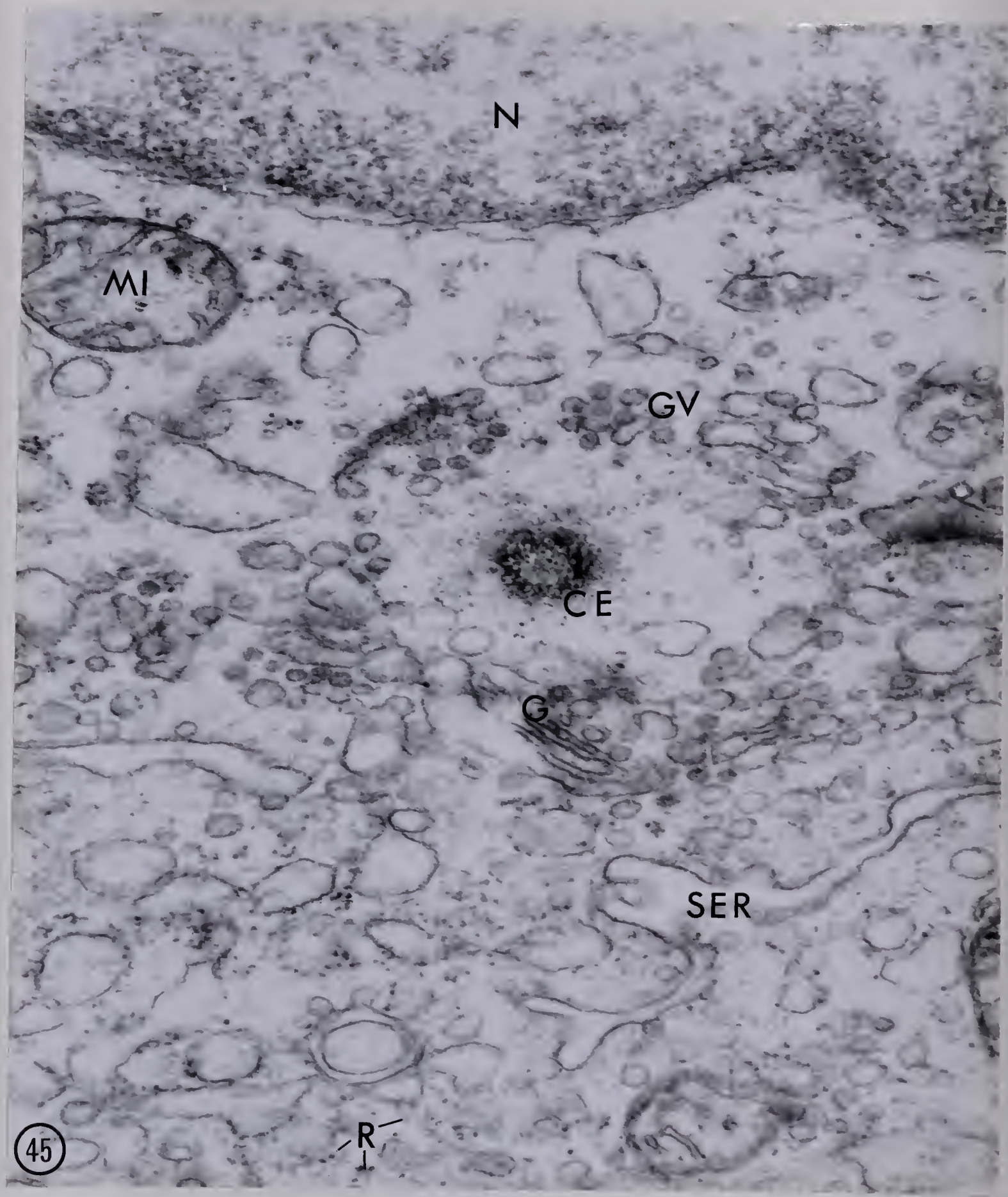

N
MI
GV
CE
G
SER
R
45

Fig. 46. Electron micrograph demonstrating a section of a valve. Two layers of endothelium separated by a dense avascular connective tissue.

CF: Collagen fibers

Glutaraldehyde - Osmium fixation, maraglas embedding, potassium permanganate and lead citrate staining. X 10.500.

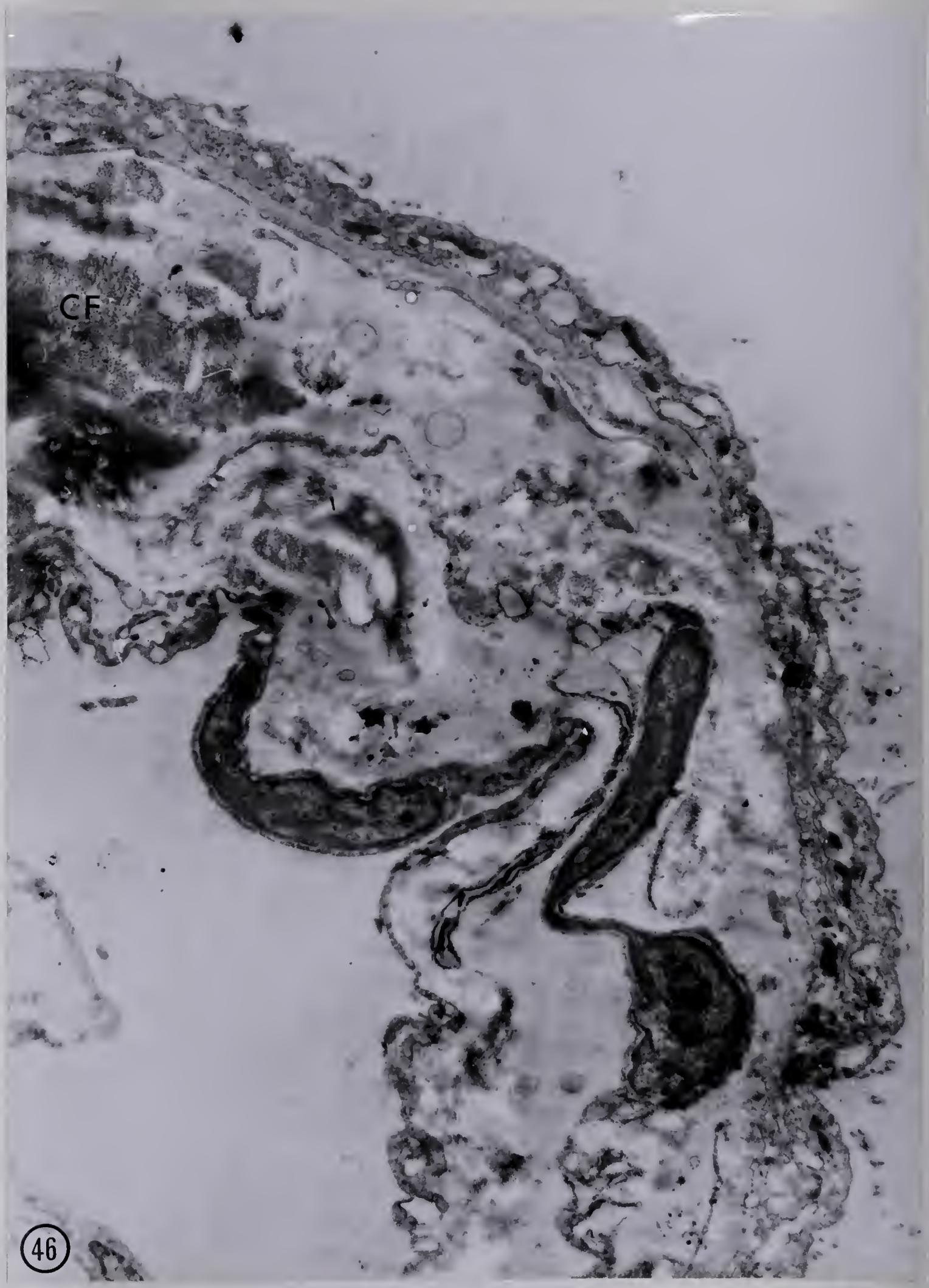
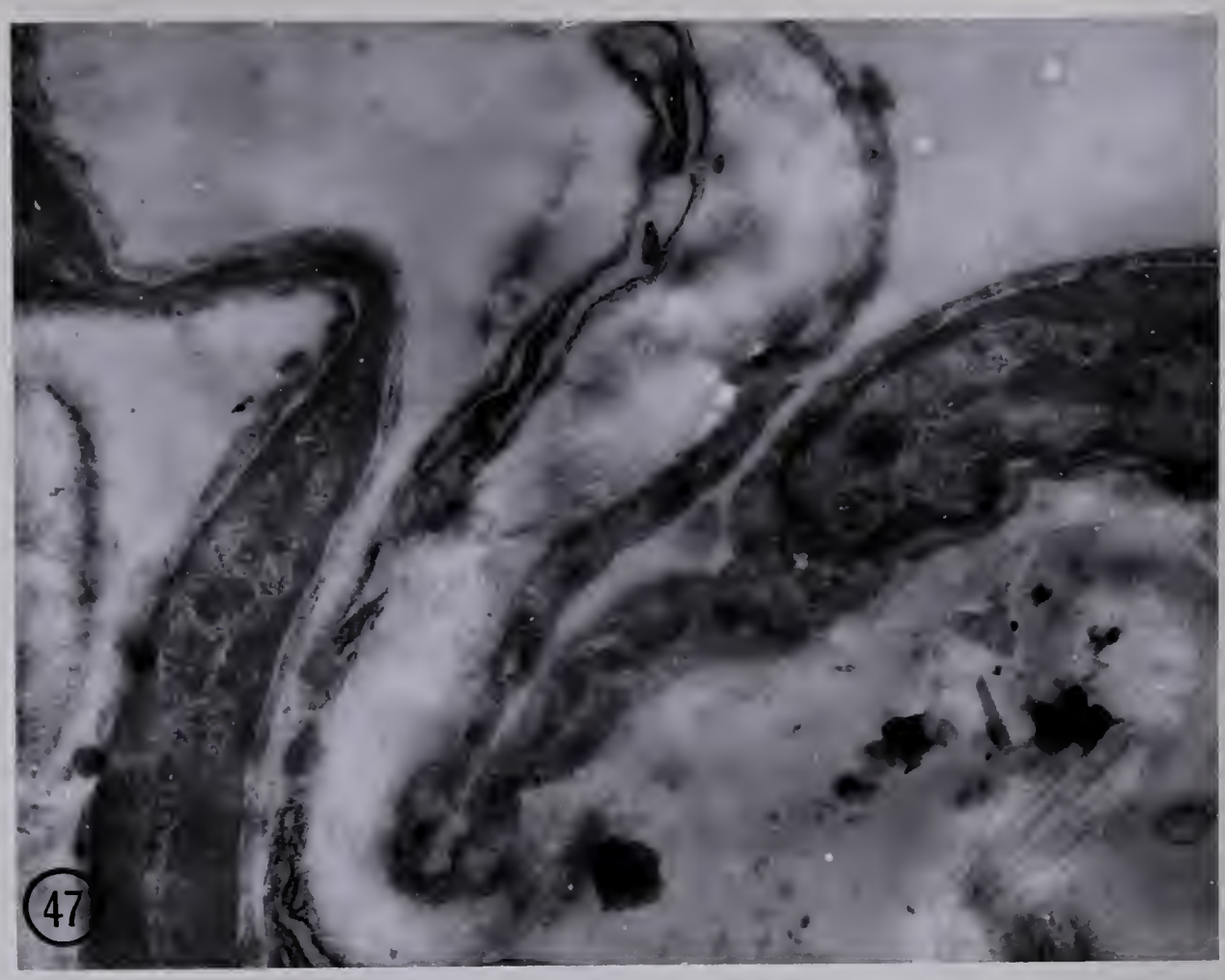

CF
46

Fig. 47. Electron micrograph from the same field as that displayed in fig. 46. The collagen fibers often run parallel to the endothelial cells but deeper within the tissue the fibers are more irregularly oriented. X 22000.

Fig. 48. Electron micrograph displaying two adjacent endothelial cells of a valve and its subendothelial layer. Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 22.000.


47

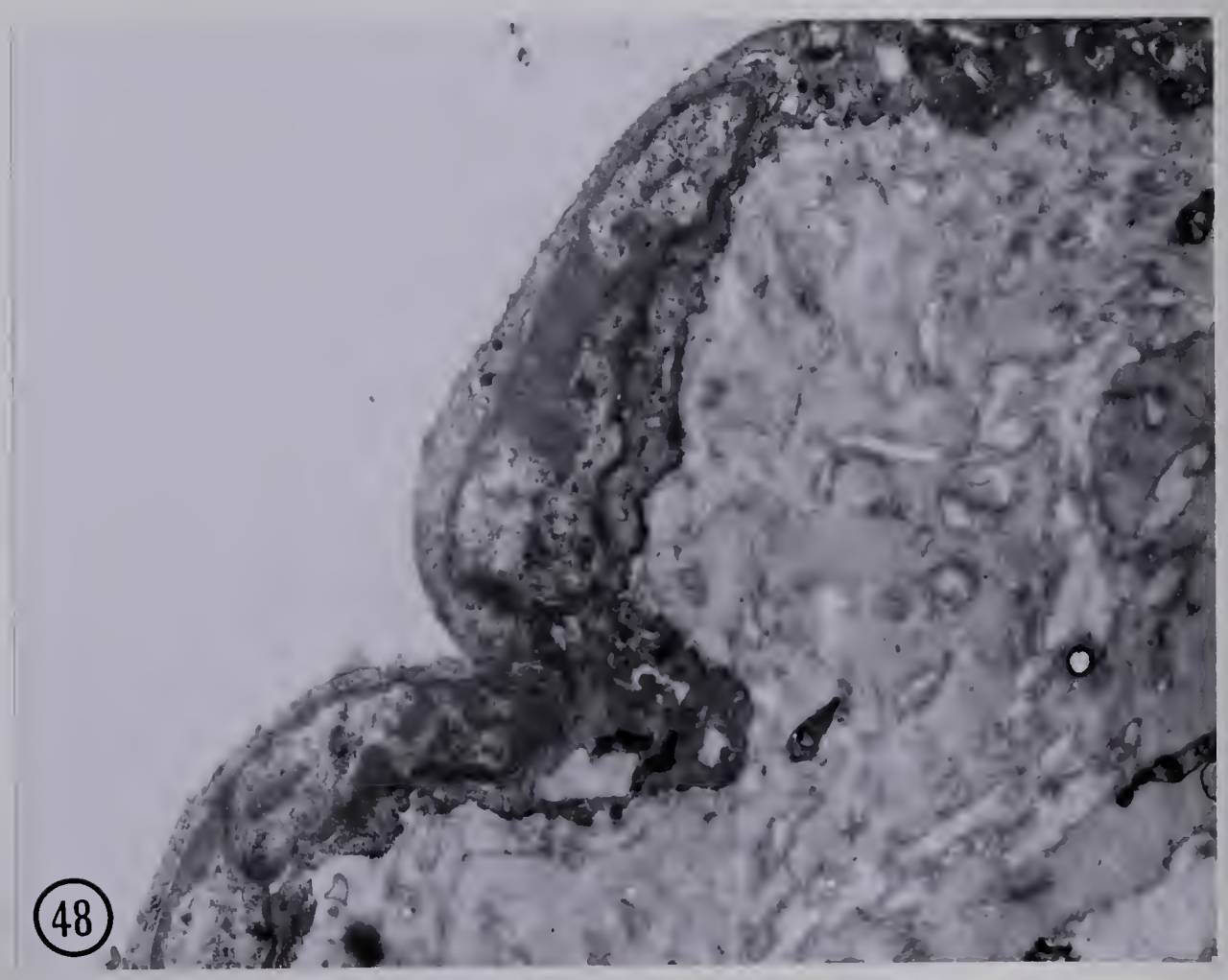
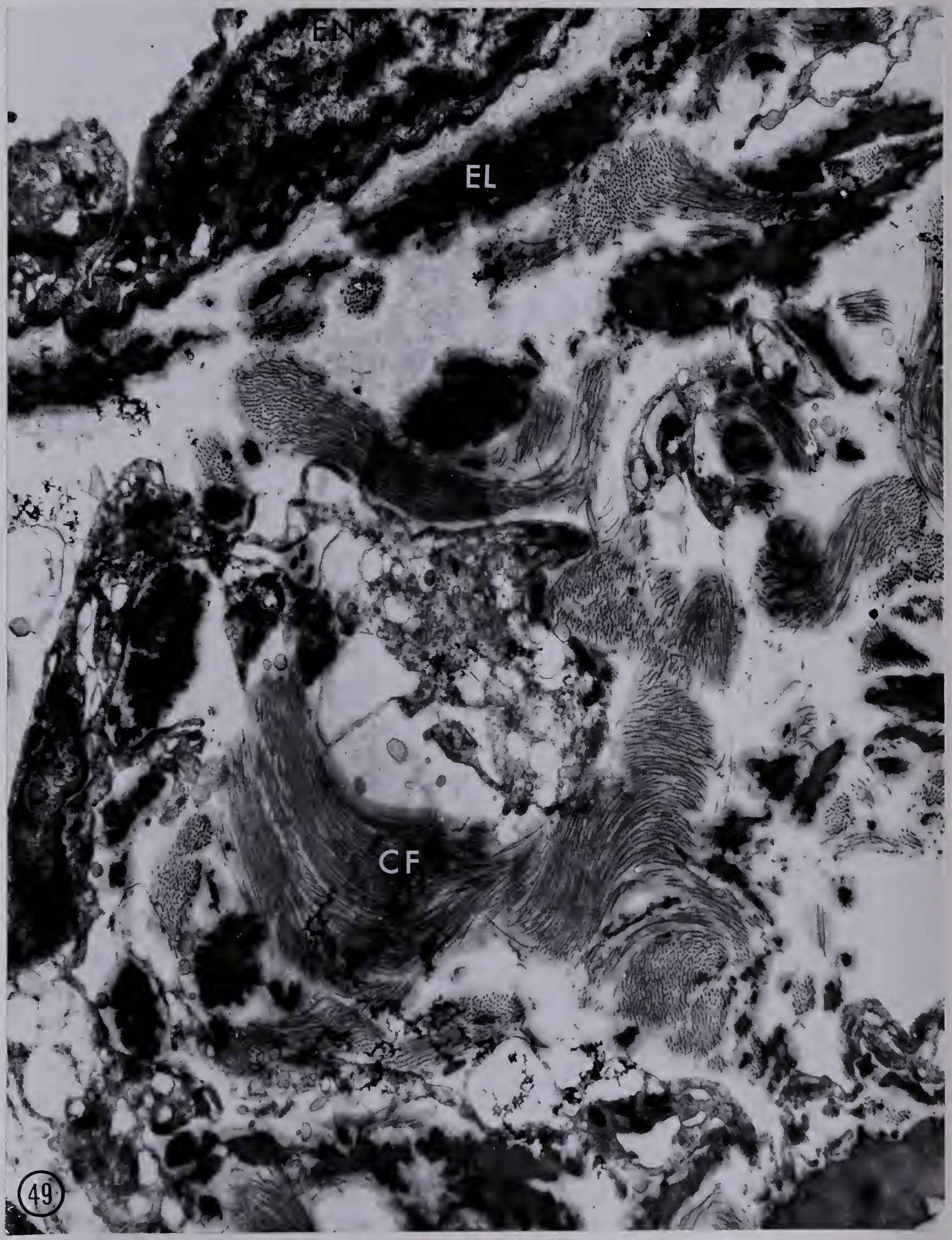

48

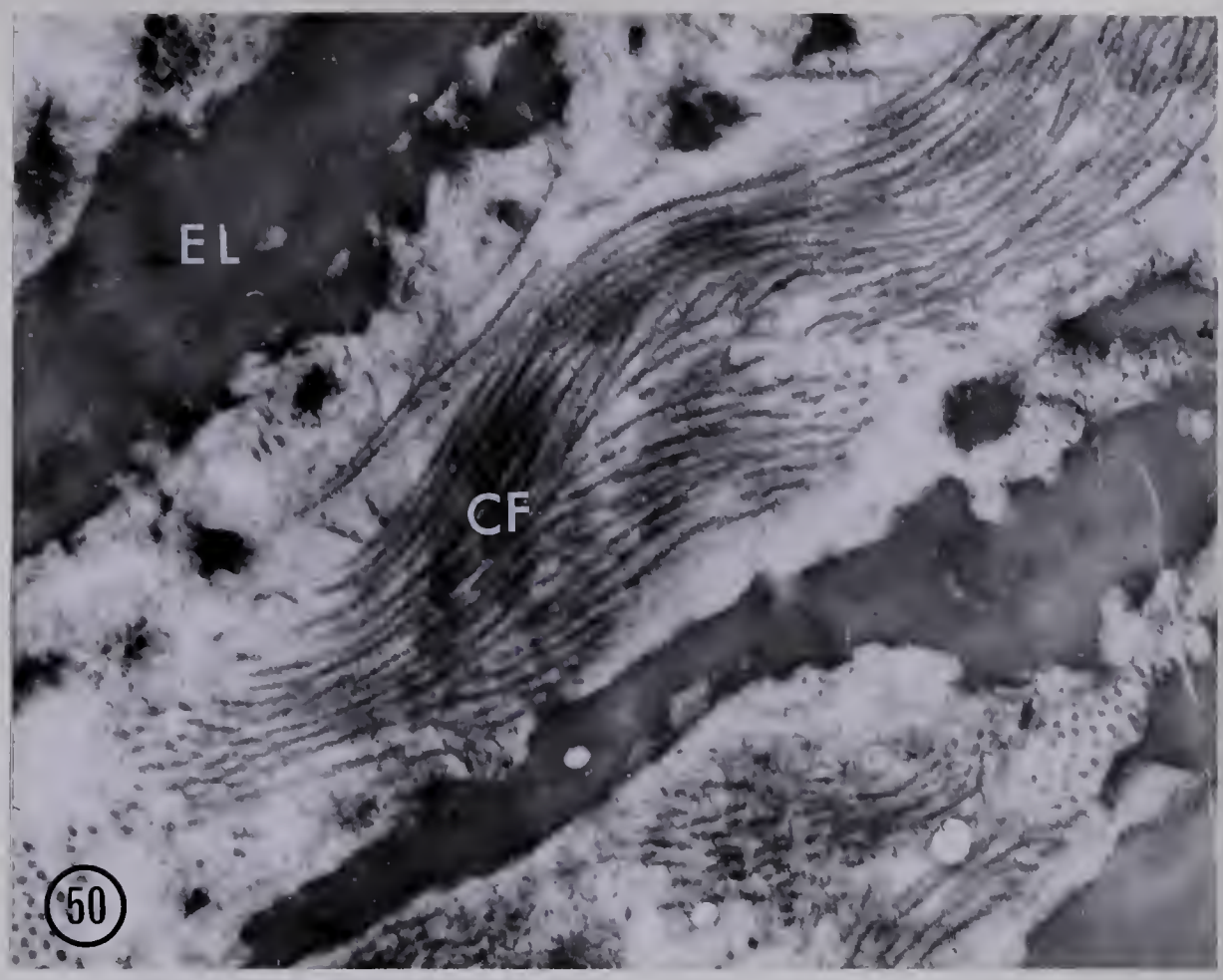
Fig. 49. Electron micrograph from the fibrous ring at the junction between the aorta and the ventricular wall. Elastic lamina (EL) is discontinuous. The elastic plates and the collagen fibers (CF) are most irregularly embedded in the ground substance. Fibroblasts are scarce. The plasma membranes of the endothelial cells (EN) are often found invaginated along the base and the nuclei are indented. Glutaraldehyde Osmium fixation, maraglas embedding, potassium permanganate and lead citrate staining. X 15000.



49

Fig. 50. Electron micrograph from a section of the aorta near the junction displayed in figure 49. The elastic lamina (EL) and the collagen fibers (CF) run reasonably parallel to each other in the ground substance.
Glutaraldehyde - Osmium fixation, maraglas embedding, ruthenium red and lead citrate stain. X 28000.

Fig. 51. Electron micrograph showing pinocytotic vesicles along the base of an endothelial cell close to the fibrous ring, in the ventricle. Periodicity in the collagen unit fibrils can be seen.
Glutaraldehyde - Osmium fixative, maraglas embedding and lead citrate staining. X 68000.


EL
CF
50

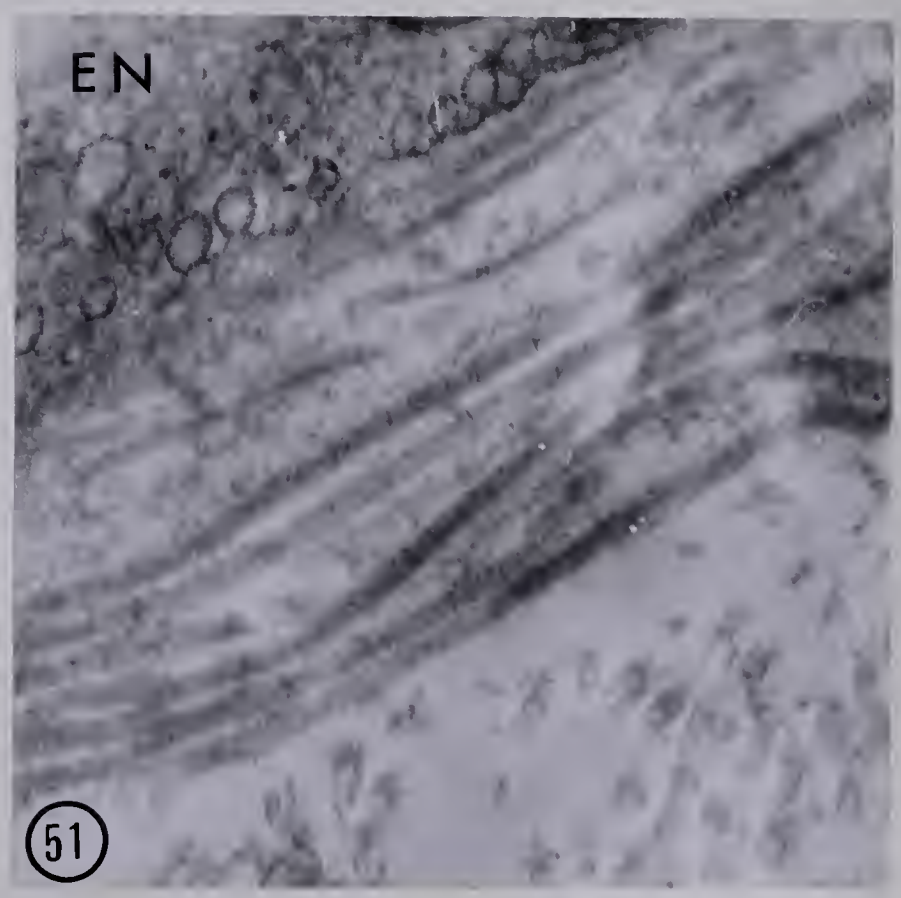

EN
51

Fig. 52. Electron micrograph of endocardium close to the junction between the aorta and the ventricular wall. Only small fragments of elastic material (EL) are found along the endothelial cells (EN). Collagen fibers are present but the fibrils are not as densly packed as they appear to be in the fibrous ring.

F: Fibroblast

Glutaraldehyde - Osmium fixation, maraglas embedding and lead citrate staining. X 15000.

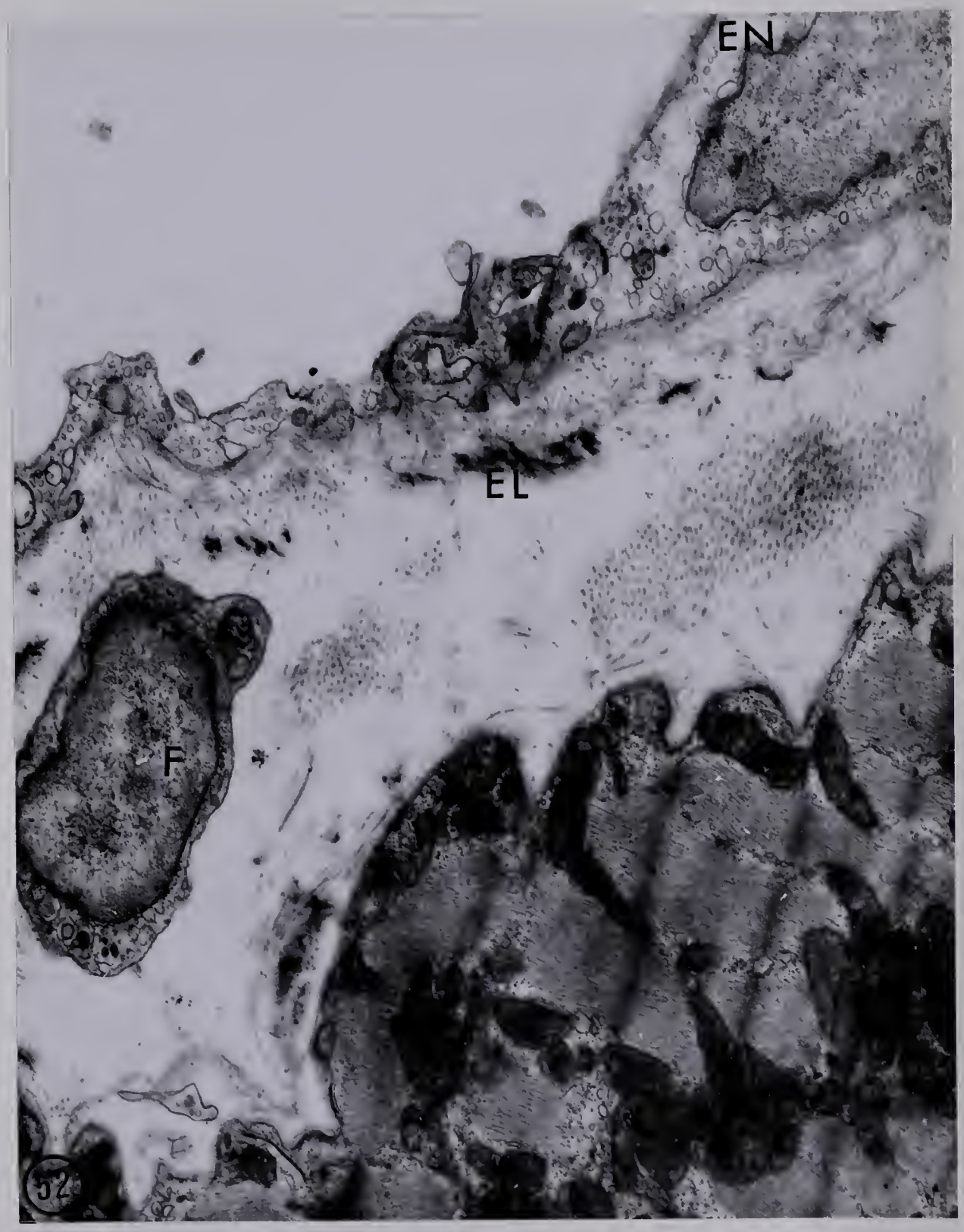

EN
EL
F
32

Fig. 53. Photomicrograph from the interventricular septum.

EN: Endothelium

MC: Myocardium

SL: Subendothelial layer

Formalin fixation, hematoxylin - eosin staining. X 240.

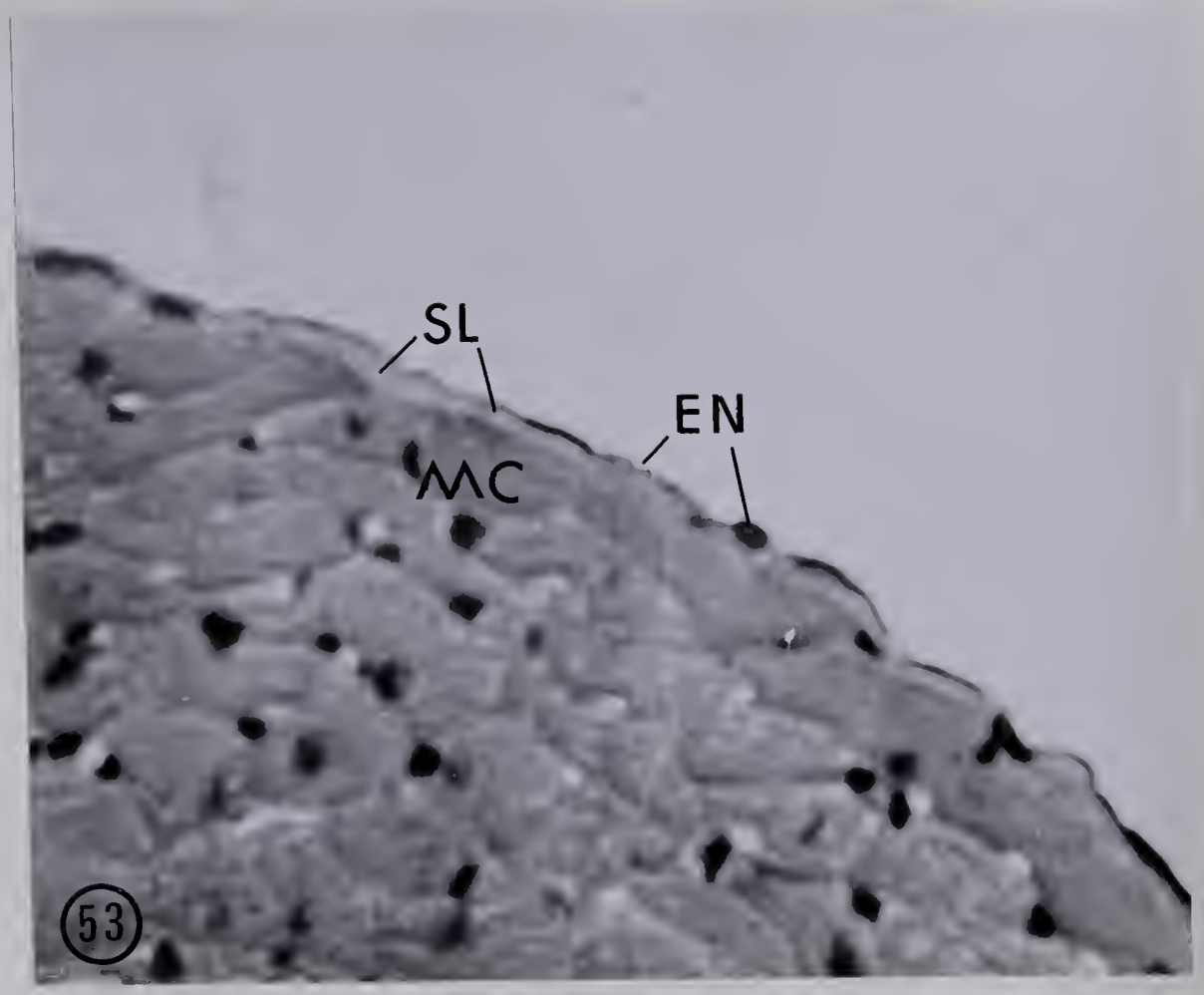
Fig. 54. Photomicrograph from the lateral wall of the ventricle.

EN: Endocardium

MC: Myocardium

SL: Subendothelial layer

Formalin fixation, hematoxylin - eosin staining. X 240.


SL
EN
MC
53

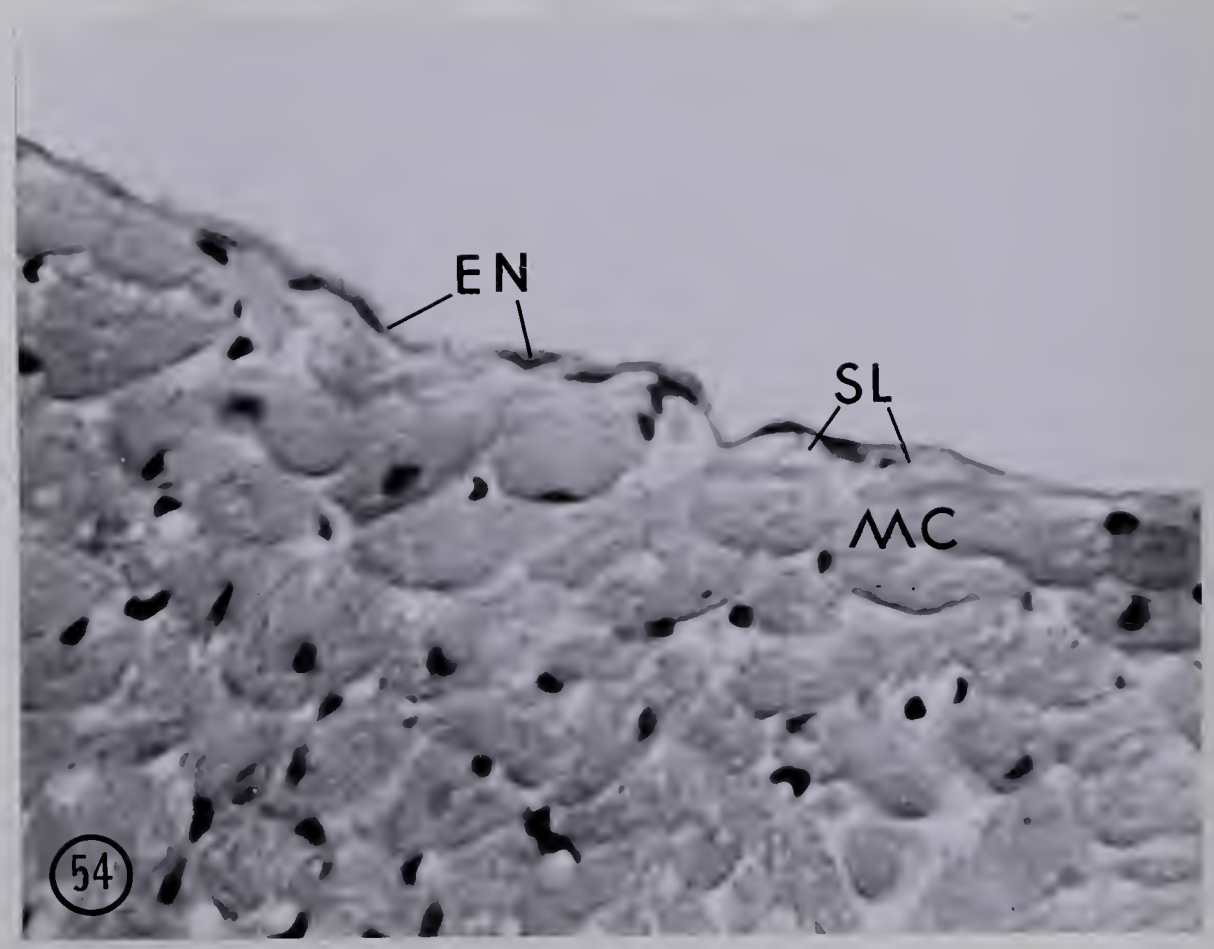

EN
SL
MC
54

Fig. 55. Photomicrograph from the atrium.

EN: Endothelium

SL: Subendothelial layer

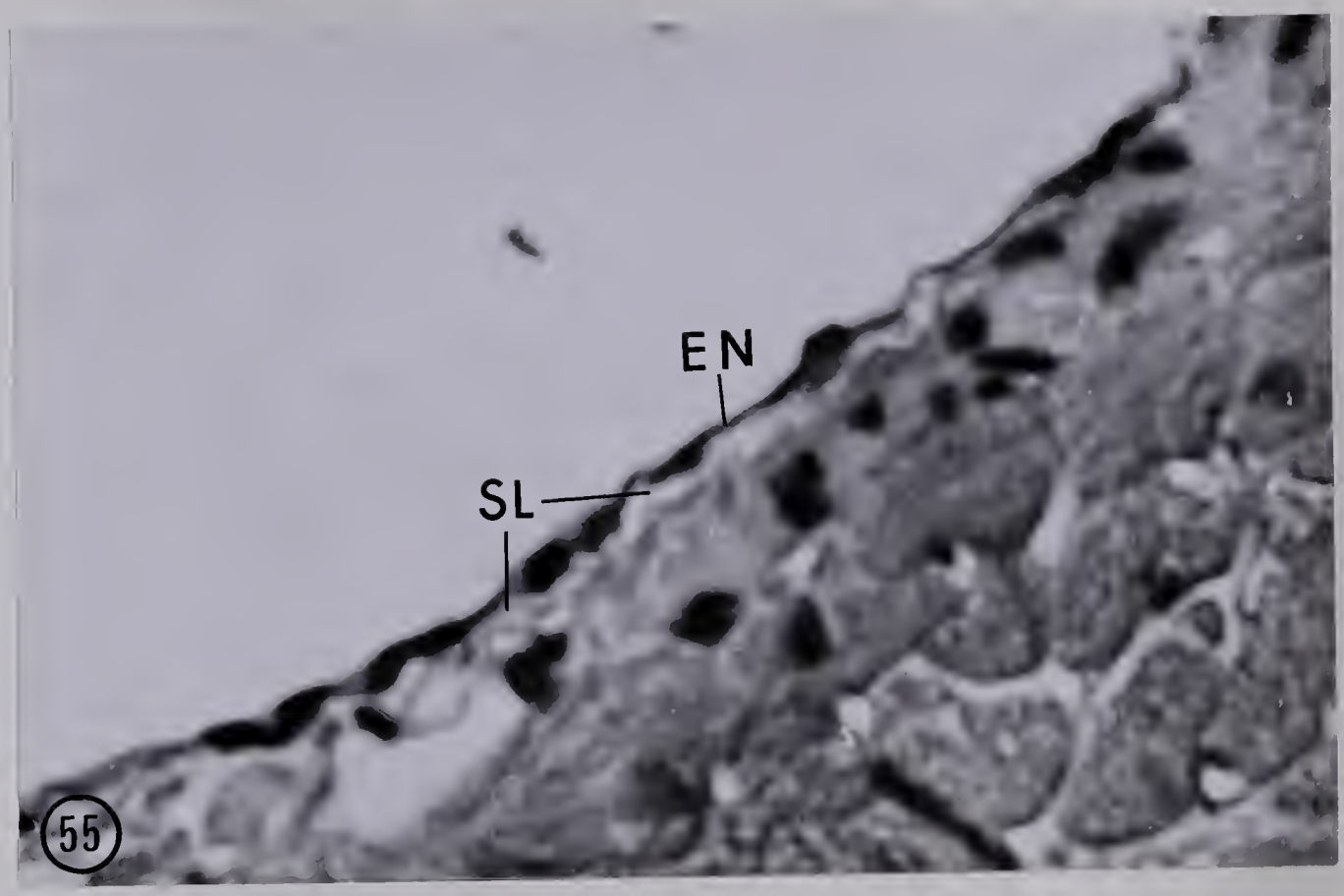
Formalin fixation, hematoxylin - eosin staining. X 300.

Fig. 56. Photomicrograph from the atrium.

EN: Endothelium

SL: Subendothelial layer

Formalin fixation, hematoxylin - eosin staining. X 240.


EN
SL
55

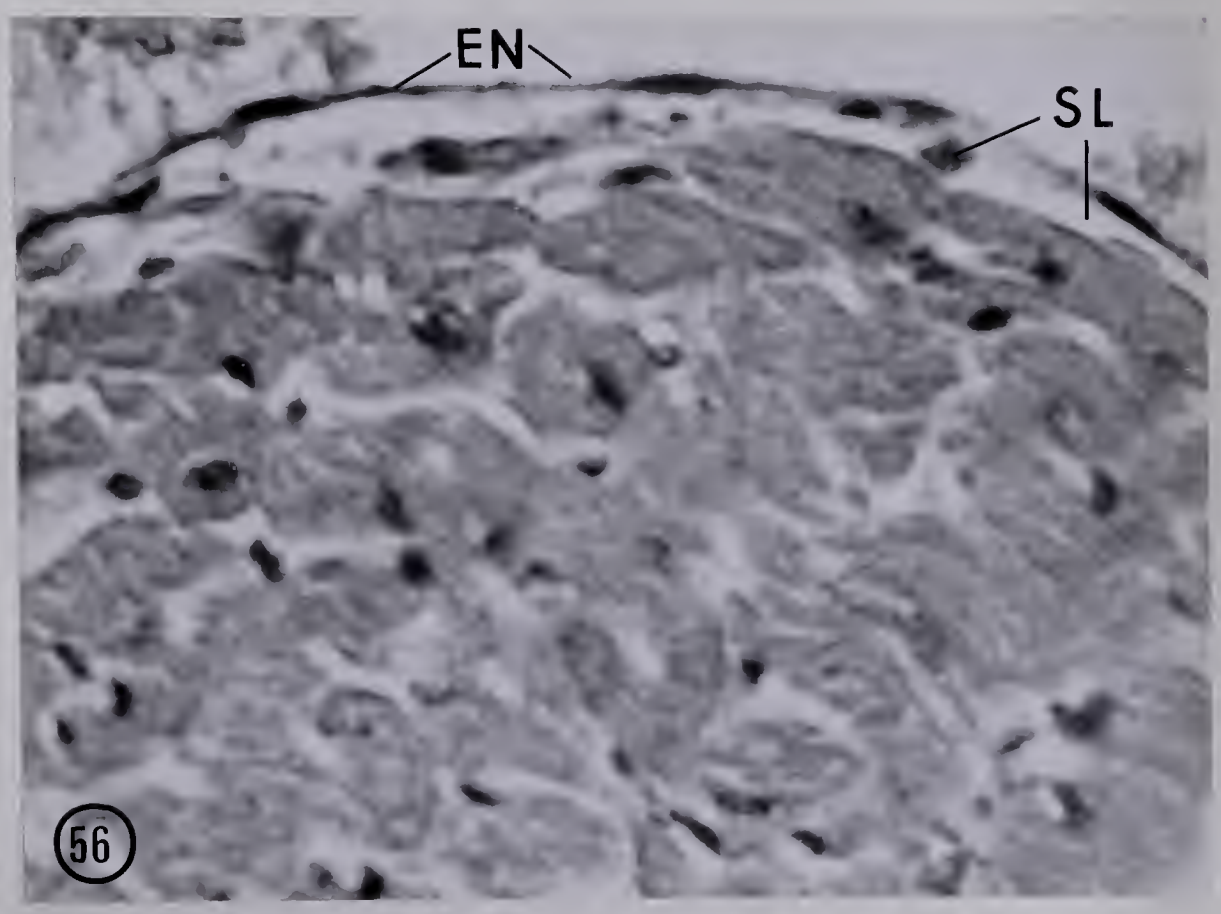

EN
SL
56

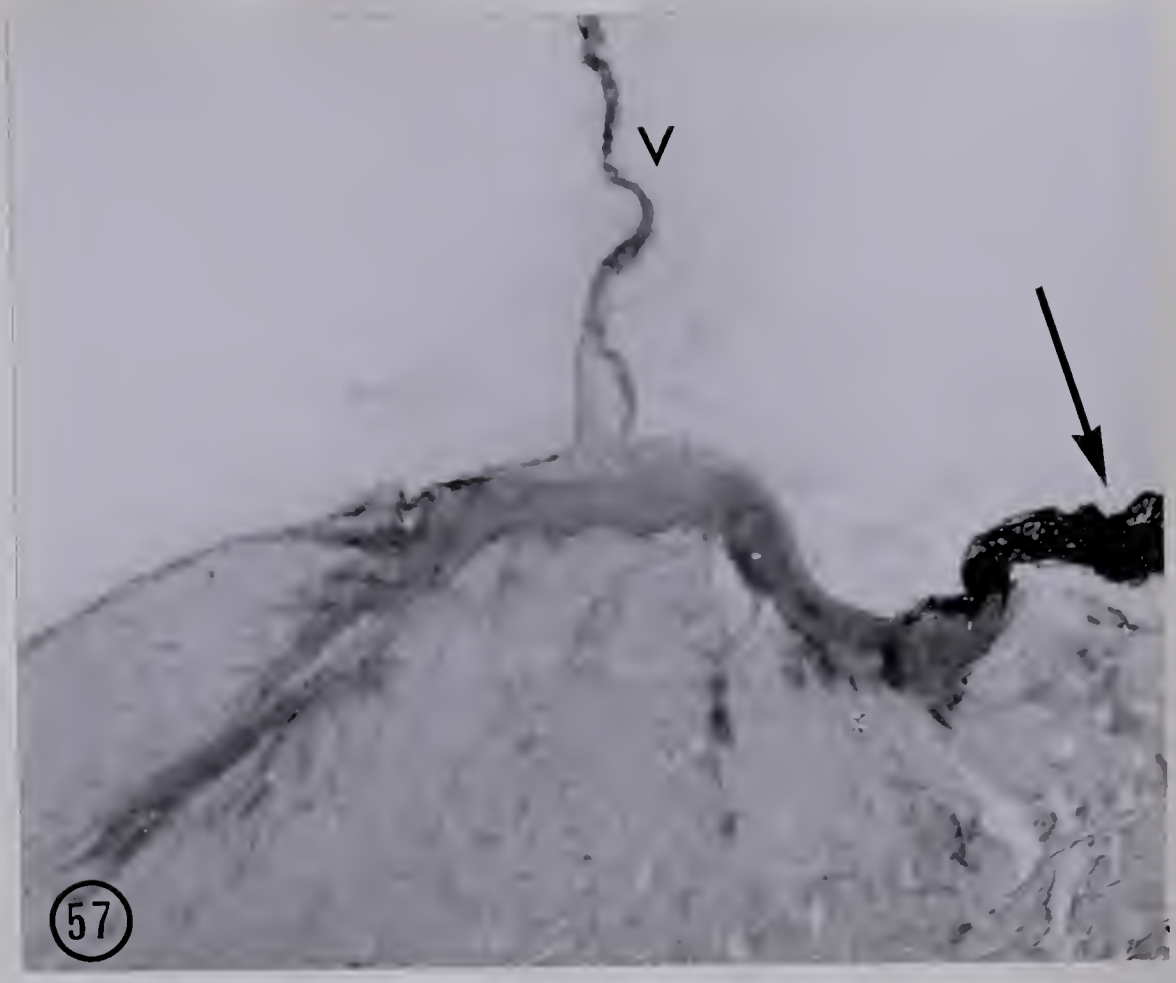
Fig. 57. Photomicrograph showing the junction between the aorta and the ventricle.

V: Valve

Zenker's fixation, Weigert's resorcein fuchsin elastic stain.

X 150

Fig. 58. Photomicrograph from the same region as illustrated in fig. 57. X 220.


V
57


58